[CONTRIBUTION FROM THE BIOLOGICAL LABORATORIES, HARVARD UNIVERSITY]

Raman Spectra of Amino Acids and Related Compounds. IX. Ionization and Deuterium Substitution in Glycine, Alanine and β-Alanine[1,2,3]

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Raman spectra have been determined for glycine, α-alanine and β-alanine in the form of the anion, dipolar ion and cation, both in ordinary aqueous solution and also in deuterium oxide after complete exchange of all H for D atoms in the amino and carboxyl groups. Certain major points are as follows: (1) The N–H stretching frequencies of the uncharged amino group, near 3305 and 3370, are shifted to 2435 and 2500 on deuteration. The N–D stretching frequency of the charged ammonium group is at 2175–2200; the corresponding N–H frequency is obscured by the C–H vibrations. The N–H bending frequencies are discussed, but are more difficult to assign. (2) The C–H stretching and bending frequencies are discussed in detail. The most unusual feature observed is that, in glycine and β-alanine, the sharp distinct lines between 2900 and 3020, observed in the cation and dipolar ion, are fused in the anion, and displaced downward into one broad strong line near 2930. This feature of the anion spectra is independent of deuteration and is not observed in α-alanine. (3) The characteristic spectral changes on ionization of the carboxyl group are similar to those of carboxylic acids in general, and the frequencies involved are nearly unchanged by deuteration. The asymmetrical stretching frequency of the ionized $-COO^-$ group, between 1570 and 1620, stands out clearly in the D_2O solution. (4) Certain skeletal frequencies in the region 800–1000 cm.$^{-1}$ are markedly affected by ionization and deuteration. One strong line near 970 in the β-alanine spectrum vanishes when this amino acid is studied in the crystalline state; presumably it corresponds to a vibration of a configuration which can be assumed in the solution, but is "frozen out" due to the inhibition of free rotation in the crystal.

The effect of ionization of the carboxyl[4] and amino groups[5] on the Raman spectra of amino acids has been investigated previously. The effects of deuterium substitution in the amino group of some simple amines also have been studied.[6] In the present communication we report the effects of ionization, and of deuterium substitution in the amino group, on the Raman spectra of three simple amino acids: glycine, alanine and β-alanine. Each amino acid can be studied in three forms—as the anion ($H_2N{\cdot}CHR{\cdot}COO^-$), the dipolar ion ($^+H_3N{\cdot}CHR{\cdot}COO^-$) and the cation ($^+H_3N{\cdot}CHR{\cdot}COOH$). On treatment with deuterium oxide, the hydrogen atoms on the amino and carboxyl groups are replaced by deuterium, those in the –CHR– group remaining unchanged. We have studied the three forms of each amino acid in ordinary aqueous solution, and also in D_2O after replacement of all exchangeable hydrogens by deuterium. Some observations of the Raman spectra of these amino acids in the solid state also are reported.

Less complete Raman spectra of glycine,[4] α-alanine[4] and β-alanine,[7] in acid and neutral solution already have been reported. No previous studies, however, have been reported of the anions of α- or β-alanine or of any of the ionic forms in D_2O solutions. The present studies also were carried out with a spectrograph of much higher dispersion and resolving power than that used in the earlier work.

Observations on the infrared spectra of these amino acids in deuterium oxide solution have been made by Gore, *et al.*,[8a] and by Lenormant,[8b] and the correlation of their measurements with ours is discussed below. Infrared studies in ordinary aqueous solution are rendered extremely difficult by the strong absorption of the water used as solvent. Raman spectra, on the other hand, are readily obtained using either H_2O or D_2O as solvent, and a wider range of conditions can be observed, in the attempt to correlate vibrational frequencies with structure.

Experimental Methods

The Raman spectra of glycine, its hydrochloride and their deuterated derivatives, were first studied by one of us (I. H. S.) using the Hilger spectrograph employed in previous studies of this series. The later studies, including a repetition and extension of the early measurements on glycine, were carried out using a three-prism glass spectrograph manufactured by the Applied Research Laboratories, Glendale, California. Details of the procedure have been discussed in the preceding paper.[1]

The amino acids used were obtained from the California Foundation for Biochemical Research and from the Mann Laboratories. The materials were of high chemical purity but sometimes contained small amounts of colored impurities, which were removed by crystallizing the amino acids from hot concentrated aqueous solutions by slow cooling and sometimes by addition of ethanol. Solutions of the amino acids, their hydrochlorides and their sodium salts were made up by procedures previously described.[5,6]

The deuterium oxide for preparing the N-deuterated amino acids was obtained from the Stuart Oxygen Company, San Francisco, California, on recommendation of the Atomic Energy Commission. Preparation of the N-deuterated amino acids was carried out by dissolving the dry crystalline amino acids in deuterium oxide, evaporating it off *in vacuo* over phosphorus pentoxide, and repeating the process until calculation indicated that the ratio of exchangeable D to H in the solution was 98 to 1 or greater. In the final solutions of amino acids in deuterium oxide the Raman water band of ordinary water above 3200 cm.$^{-1}$ was undetectable. Isoelectric amino acids, dissolved in D_2O, were converted to the hydrochlorides, for study of the amino acid cations, by passing DCl gas into the solution; the DCl was generated from the interaction of D_2O and benzoyl chloride, as described by Brown and Groot.[9] Solutions of the sodium salts

(1) For the preceding paper of this series, see D. Garfinkel and J. T. Edsall, THIS JOURNAL, **80**, 3807 (1958).

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(3) The studies reported here are taken in part from portions of the Ph.D. thesis of David Garfinkel (1955) and of the honors theses in Biochemical Sciences of I. H. Scheinberg (1940) and Ronald E. S. Iavazzo (1956).

(4) J. T. Edsall, *J. Chem. Phys.*, **4**, 1 (1936); **5**, 508 (1937).

(5) J. T. Edsall, *ibid.*, **5**, 225 (1937).

(6) J. T. Edsall and H. Scheinberg, *ibid.*, **8**, 520 (1940).

(7) J. T. Edsall, J. W. Otvos and A. Rich, THIS JOURNAL, **72**, 474 (1950).

(8) (a) R. C. Gore, R. B. Barnes and E. Petersen, *Anal. Chem.*, **21**, 382 (1949); (b) H. Lenormant, *J. chim. phys.*, **49**, 635 (1952).

(9) H. C. Brown and C. Groot, THIS JOURNAL, **64**, 2223 (1942).

of the amino acids in D_2O, for the study of the spectra of the amino acid anions, were prepared by adding concentrated NaOD solution to the solution of the isoelectric amino acid in D_2O.

The solutions of the neutral amino acids, and of their hydrochlorides, were clarified by treatment with purified charcoal and filtration through Whatman filter paper into the Raman tube. The alkaline solutions could not be treated with charcoal, which liberates traces of fluorescent impurities into the solution when the *p*H is much above 7. Filtration through a Millipore filter[10] proved helpful in several instances in clarifying these alkaline solutions, but the Raman spectra of alkaline solutions of amino acids always have shown more background and have been inferior in quality to those obtained from neutral or acid solutions. Nevertheless quite detailed spectra can be obtained from such solutions, as the data recorded in the tables of this paper indicate.

Glycine was studied at a concentration of approximately 2.7 molar, α-alanine at approximately 1.5 molar, β-alanine at 4 molar; the solution being not far from saturated in each case. The hydrochlorides were studied at similar concentrations. The solutions of the sodium salts were somewhat more dilute, due to the dilution of the amino acid by the added NaOH (or NaOD, for solutions in D_2O).

Experimental Results

The observed spectra, in the region below 2000 cm.$^{-1}$, are recorded in Tables I, II and III for glycine, α-alanine and β-alanine, respectively. The spectra above 2000 cm.$^{-1}$, which represent the stretching frequencies involving C–H, C–D, N–H and N–D bonds, are listed separately in Tables IV and VI. These two tables, together with Tables V and VII, serve to indicate the assignments of many of the observed frequencies. Additional comment, with tentative assignment of some of the other frequencies, is given below.

TABLE I[a]

GLYCINE. SPECTRA IN REGION BELOW 2000 CM.$^{-1}$

Spectra in H_2O			Spectra in D_2O		
NH_2	NH_3^+	NH_3^+	ND_2	ND_3^+	ND_3^+
CH_2	CH_2	CH_2	CH_2	CH_2	CH_2
COO^-	COO^-	$COOH$	COO^-	COO^-	$COOD$
512(3b)	511(3b)	503(2b)	482(vw)	500(1b)	490(2b)
	585(1b)	577(vw)		595(vw)	
	667(1b)	657(vw)	650(vw)	640(1)	840(vw)
896(6)P	896(7)P	871(7)P	834(2)	840(3)P	774(4b)P
		912(vw)			810(vw)
			923(7)	912(1)	900(vw)
			970(2)	964(5)P	945(3)
				1000(2)	1005(4)
	1031(3)	1043(4)	1062(3b)		1040(1)
1106(2b)	1125(1vb)	1120(1vb)P			1075(vw)
					1170(1)
			1205(3b)	1200(1b)	1210(3)
1166(1b)	1197(vw)	1259(3)		1273(4)	1278(6)
1313(3)	1329(8)P	1316(3)	1317(5)	1320(6)P	
1400(10)	1411(8b)P?		1408(10)	1409(8)P	1390(vw)
1434(6b)	1441(3)	1433(6)	1448(3)	1438(4)	1430(2)
		1743(6b)P	1566(2b)	1623(3b)	1735(3b)P

[a] All values in cm.$^{-1}$. Figures in parentheses denote relative intensities. The symbol "b" denotes a broad line; "vw" denotes a very weak or questionable line. All spectra showed the characteristic bands of H_2O or D_2O, which are not listed here. Polarization studies were made on glycine, glycine hydrochloride and their deuterated derivatives. Lines which were definitely or probably polarized are denoted by P or P?. Frequencies in the region 2000 cm.$^{-1}$ are given separately, in Tables IV and V. The arrangement of spectra in parallel columns in Tables I, II and III, is adopted to permit ready comparison between the data for the different ionic forms, deuterated or undeuterated. Frequencies of similar numerical values are placed in corresponding, or nearly corresponding, positions in the different columns; this does not imply that they must correspond to similar modes of vibration. Where such correspondences are believed to exist, this is indicated in Tables IV–VII, inclusive, and in the discussion in the text. In Tables I, II and III no frequencies between 1510 and 1700 are listed for the solutions in H_2O. All those solutions show the water band which centers in the region 1620–1640, and there are probably frequencies due to the solute which lie in, or closely adjacent to, the water band on the low frequency side (1560–1620). These lines are not strong, however, and cannot yet be clearly distinguished. See discussion in text.

(10) Millipore Filter Corporation, Watertown, Mass.

TABLE II[a,b]

α-ALANINE. SPECTRA IN REGION BELOW 2000 CM.$^{-1}$

Spectra in H_2O			Spectra in D_2O		
NH_2	NH_3^+	NH_3^+	ND_2	ND_3^+	ND_3^+
$HC-CH_3$	$HC-CH_3$	$HC-CH_3$	$HC-CH_3$	$HC-CH_3$	$HC-CH_3$
COO^-	COO^-	$COOH$	COO^-	COO^-	$COOD$
	398(1b)	409(vw)			
	460(1b)			453(2b)	
538(5)	527(4)	520(1)	540(1)	515(2b)	512(2)
					576(1)
			647(vwb)		611(0)
			715(vw)	765(vw)	748(2)
812(1)	771(2)	745(3)	768(vw)	806(6b)	786(2b)
849(9)	843(8)	819(7)	823(2)		834(0)
	918(4)	918(4)	878(1)	874(2)	886(1)
971(4)	999(6b)	1006(4)	932(1)	917(4)	914(1)
1019(6)	1111(6b)	1119(4)		1000(vw)	
1159(3)	1125(2)		1050(5)	1052(4)	1066(4)
			1104(2)		1091(3)
			1195(2)	1210(3b)	1150(2)
		1624(vw)	1234(2)		1200(2b)
1280(1)	1298(3)		1287(4)	1292(4)	
	1350(7)	1321(1b)	1330(3)	1336(7)	1331(2)
1359(2)	1373(3)	1363(1b)	1365(1)	1373(4)	
1413(8)	1410(8)	1433(vw)	1408(8)	1414(8)	1406(3)
1455(7)	1456(7)	1458(5)	1453(6)	1459(6)	1460(8)
			1563(2b)	1612(2)	
		1726(4b)		1660(1)	1738(6b)

[a] For explanation of figures in parentheses, refer to footnote, Table I. [b] Frequencies in the region above 2000 cm.$^{-1}$ are given separately, in Tables IV and V. It is important also to consult the footnote to Table I, which applies equally to the data given here, except that no polarization studies were made on α-alanine or β-alanine.

Discussion

Nitrogen–Hydrogen Stretching Frequencies (Table IV).—These are assigned readily in the light of extensive experience in the study of amines, amino acids and other related compounds.[5,6,11,12] The uncharged amino group of the amino acid anions gives rise to two lines, one of moderate intensity near 3370, the other of high intensity near 3305. When hydrogen is replaced by deuterium, these frequencies are displaced to 2500 and 2435, respectively, the frequency ratio of the N–H to the corresponding N–D vibration being consistently 1.35–1.36, as in other compounds in which the $-NH_2$ and $-ND_2$ group vibrations have been compared (see for instance reference 6, Table I). The frequencies in the amino acid anions are consistently 10 to 20 cm.$^{-1}$ lower than those of CH_3NH_2 and CH_3ND_2 (see ref. 6). It appears clear[6,11,12] that the lower of the two frequencies

(11) L. J. Bellamy, "The Infra-red Spectra of Complex Molecules," Methuen and Co., Ltd., London, John Wiley and Sons, Inc., New York, N. Y., 1954.

(12) R. N. Jones and C. Sandorfy, "The Application of Infrared and Raman Spectrometry to the Elucidation of Molecular Structure," in "Technique of Organic Chemistry" (A. Weissberger, editor), Vol. IX, "Chemical Applications of Spectroscopy" (W. West, editor), Interscience Publishers, New York, N. Y., 1956.

TABLE III[a,b]

β-ALANINE. SPECTRA IN REGION BELOW 2000 CM.$^{-1}$

Spectra in H_2O			Spectra in D_2O		
NH_2 $(CH_2)_2$ COO^-	NH_3^+ $(CH_2)_2$ COO^-	NH_3^+ $(CH_2)_2$ $COOH$	ND_2 $(CH_2)_2$ COO^-	ND_3^+ $(CH_2)_2$ COO^-	ND_3^+ $(CH_2)_2$ $COOD$
	365(2b)	385(2b)	341(vw)	360(1b)	350(1b)
470(1b)	488(1b)	489(wb)	401(vw)	490(2b)	480(1b)
527(vw)	519(wb)	512(1b)			
607(vw)	595(2b)	574(1b)		600(1b)	570(vw)
661(1b)		667(vwb)		650(vw)	640(vw)
852(3)	843(3)	817(7)		745(1b)	730(wb)
				825(1b)	810(6)
876(4)	866(8)	864(8)	868(1)	850(5)	845(5)
		906(3)	900(5)	896(6)	907(1)
933(5)	928(7)	926(3)	936(4)	925(2)	
990(vw)	970(3)	965(4)	967(vw)	973(4)	971(3)
1024(4)	1034(2)	1033(2)	1006(2b)	1000(2)	
1071(3b)	1047(5)	1050(6)	1033(1)	1023(3)	1019(4)
			1081(vw)	1087(4b)	1093(4)
1142(1)	1120(1vb)	1125(1b)	1169(1b)	1131(1vb)	1140(3vb)
		1167(vw)		1181(1vb)	1170(3vb)
1220(2b)		1222(1b)	1206(2b)	1222(3b)	1190(1)
					1222(3vb)
	1257(4)	1259(6)	1270(vw)	1274(7)	
1311(3b)	1294(5)		1316(4)	1304(3)	1300(7)
	1327(8)	1322(7)		1315(8)	
1354(2)	1382(3)	1392(vw)	1363(0)	1346(1)	1356(1)
				1373(3)	
1406(10)	1405(9)	1406(8)	1406(10)	1398(7)	
				1417(4)	1407(7)
1457(6)	1460(6)	1462(7)	1462(2)	1460(5)	1461(7)
		1504(w)		1523(vw)	
			1560(2b)	1570(4b)	
		1723(5b)			1707(6b)

[a] For explanation of figures in parentheses refer to footnote, Table I. [b] Frequencies in the region above 2000 cm.$^{-1}$ are given separately, in Tables IV and V. It is important also to consult the footnote to Table I, which applies equally to the data given here, except that no polarization studies were made on α- or β-alanine.

expected line near 2970 is masked by the sharper and far more intense C–H vibrations. No other line corresponding to N–H (or N–D) stretching in the charged ammonium group has been found. It is notable that this line is much weaker and broader than the lines characteristic of the uncharged amino group and is lower in frequency by several hundred cm.$^{-1}$, which corresponds to the much stronger hydrogen bonding between the surrounding water molecules and the charged ammonium group than is found in the uncharged amino group.

The Nitrogen–Hydrogen Deformation Frequencies.—These are more uncertain than the stretching frequencies. Edsall and Scheinberg[6] noted a broad diffuse line near 1620 in $CH_3NH_3^+$ and other compounds containing the $-NH_3^+$ group, and a probably corresponding line near 1200 in the corresponding compounds containing the $-ND_3^+$ group. These lines appeared too intense to be assigned to the broad weak lines arising from the solvent (near 1640 for H_2O, and near 1210 for D_2O). It is difficult, however, to distinguish similar frequencies in the Raman spectra of these amino acids. The presence of the water band near 1640 (not recorded in Tables I, II and III) may mask the presence of other lines in this range; also the asymmetrical stretching frequency of the ionized $-COO^-$ group near 1600 (discussed below) may cause further difficulties in interpretation. On account of the presence of the water band, it is difficult to determine whether lines due to the solute are present, in ordinary water, in the region from 1570 to 1650. We believe that such lines are present, but have not recorded them in Tables I, II and III since they are in any case rather weak and difficult to identify. We plan to return to this problem later, using photoelectric recording to obtain quantitative intensity measurements of the Raman lines. For the present we make no attempt to assign N–H or N–D deformation frequencies.

TABLE IV

STRETCHING FREQUENCIES OF THE $-NH_3^+$, (ND_3^+) AND $-NH_2(ND_2)$ GROUPS

Substance	Form	$-NH_3^+$	$-ND_3^+$	$-NH_2$	$-ND_2$	$\frac{\nu(N-H)}{\nu(N-D)}$
Glycine	Anion			3376(4)	2504(4b)	1.35
				3318(8)	2434(8b)	1.36
	Dipolar ion	Probably masked by	2200(1b)			
	Cation	C–H stretching 3000	2201(2b)			
α-Alanine	Anion			3371(6)	2507(5)	1.34
				3305(8)	2438(6)	1.36
	Dipolar ion	Masked	2180(2b)			
	Cation	Masked	2170(1b)			
β-Alanine	Anion			3362(5)	2498(5b)	1.35
				3303(10)	2435(6)	1.36
	Dipolar ion	Masked	2198(5b)			
	Cation	Masked	2202(4b)			

corresponds to the symmetrical, the higher to the asymmetrical, stretching of the $-NH_2$ (or $-ND_2$) group.

In the dipolar ions and cations containing the $-ND_3^+$ group (Table IV) a broad, rather weak line consistently appears near 2175 in α-alanine, and near 2200 in glycine and β-alanine. A similar line is found[6] in the $CH_3ND_3^+$ and the $^+D_3N{\cdot}ND_2$ ions, at 2190 and 2176, respectively. In $^+H_3N{\cdot}NH_2$, a corresponding line appears at 2963, the ratio 2963/2190 being 1.36 as would be expected for an N–H stretching frequency. In the $CH_3NH_3^+$ ion and the amino acids containing the NH_3^+ group, the

Carbon–Hydrogen Vibrations, Table V: (a) Methylene Frequencies in Glycine and β-Alanine.—A "normal" CH_2 group, attached to saturated carbon atoms in a hydrocarbon, shows a bending frequency close to 1465 and two stretching frequencies,[11,12] a symmetrical one near 2850 and an asymmetrical one near 2925. In glycine the former is displaced downward, to the region 1430–1440, in all the various ionic forms, while the two stretch-

TABLE V

STRETCHING AND DEFORMATION VIBRATIONS OF $-CH_3$, $-CH_2$ AND $-CH$ GROUPS

Substance	Form		Stretching H_2O	Stretching D_2O	Deformation H_2O	Deformation D_2O
	Cation		3017(4)	3013(3)	1433(6)	1430(2)
			2974(10)	2971(10)		
Glycine	Dipolar ion		3013(3)	3011(3)	1441(3)	1438(4)
$-CH_2$			2971(10)	2969(10)		
	Anion		2940(8b)	2934(8b)	1434(6b)	1448(3)
	Cation	CH_3	3013(7)	3013(7)	1458(5)	1460(8)
		CH_3	2957(10)	2961(10)		1406(3)
		C-H	2899(4)	2909(5)	?	?
α-Alanine	Dipolar ion	CH_3	3002(7)	2997(7)	1456(7)	1459(6)
$-CH_3$		CH_3	2950(10)	2956(10)	1410(8)	1414(8)
$-CH$		C-H	2892(5)	2902(3)	?	?
	Anion	CH_3	2983(7)	2977(7)	1455(7)	1453(6)
		CH_3	2940(10)	2938(10)	1413(8)	1408(8)
		C-H	2885(5)	2886(5)	?	?
	Cation		3019(4)	3018(4)	1463(7) β-	1461(7)
			2987(10)	2983(10)	1406(8) α-	1407(7)
			2931(10)	2926(10)		
β-Alanine	Dipolar ion		3015(4)	3009(3)	1460(6) β-	1460(4)
α-CH_2			2978(10)	2976(10)	1405(9) α-	1417(4)
β-CH_2			2952(10)	2918(10)		
	Anion		2925(8b)	2926(8b)	1457(6) β-	1462(2)
					1406(10)? α-	1406(10)?

ing frequencies are much higher than "normal," at 2970 and 3015, in the cation and dipolar ion forms. The line at 2970 is far the more intense of the two and is polarized, so that it clearly corresponds to the symmetrical vibration. Removal of the positive charge on the ammonium group, to form the glycine anion, produces a large and quite unexpected alteration in these frequencies. Instead of two sharp lines there is a single very broad intense band with a maximum of intensity near 2940. Why the ionization of the amino group should affect so profoundly the vibration of the adjoining methylene group is still obscure; the deformation frequency near 1440 remains essentially unchanged. All these frequencies are independent of deuterium substitution in the amino and carboxyl hydrogens.

In β-alanine, the two methylene groups are not equivalent. Correspondingly there are two deformation frequencies, one at 1460, the other near 1410; the latter overlaps with the symmetrical stretching frequency of the ionized carboxyl group. Comparison with the spectra of amines[5,6] and of compounds containing methylene groups adjoining carbonyl or carboxyl groups[11-14] indicates that the lower frequency corresponds to the α-methylene group adjoining the carboxyl, the higher to the β-methylene group.

No sure basis exists for allocation of the three C-H stretching frequencies near 2950, 2980 and 3015 in β-alanine. They are essentially unaltered in position or intensity by ionization of the carboxyl, or by deuterium substitution in the carboxyl or ammonium group. They are all of much higher frequency than the "normal" methylene stretching frequencies referred to above. When the amino group loses its charge, these frequencies are altered even more dramatically than in glycine; only a single broad intense band, centered at 2925, remains in the β-alanine anion, and this is at a much lower frequency than any of the three lines observed in the dipolar ion and cation. The effect is the same, whether the anion contains an $-NH_2$ or an $-ND_2$ group.

The case of α-alanine is different, since it contains no methylene group, but a methyl group and a C-H linkage on the α-carbon atom. There is a strong methyl deformation frequency near 1460, and another frequency near 1410 (which, however, is missing in the undeuterated cation). This is probably a methyl group frequency also, although in the dipolar ion and anion it is overlaid by the symmetrical $-COO^-$ stretching frequency near 1400. Of the three strong frequencies near 2890, 2950 and 3000, the lowest is probably to be assigned to a stretching motion of the tertiary C-H bond, the other two to the methyl group. These frequencies are the same in the anion as in the dipolar ion or cation, in marked contrast to the situation in glycine and β-alanine.

The Vibrations of the Carboxyl Group (Table VI).—The characteristic C=O stretching frequency in the the un-ionized carboxyl group is found near 1740 in the glycine cation, at a slightly lower level in the α-alanine cation, and about 1720 in the β-alanine cation. In the deuterated form of the latter it falls[15] to 1707.

The ionized carboxyl group is known[4,11,12] to give rise to two principal stretching frequencies: an asymmetrical frequency in the region 1570–1600, and a symmetrical frequency near 1400. In the Raman spectrum, although not in infrared absorption, the latter is by far the more intense. As Tables I, II, III and VI show, it is indeed an intense line in the anionic and dipolar ion form of

(13) S. A. Francis, *J. Chem. Phys.*, **19**, 942 (1951).

(14) R. N. Jones and A. R. H. Cole, THIS JOURNAL, **74**, 5648 (1952); R. N. Jones, A. R. H. Cole and B. Nolin, *ibid.*, **74**, 5662 (1952).

(15) In the α-alanine cation (Table VI) deuteration appears to raise this frequency by 12 cm.$^{-1}$. Whether this finding is significant remains for further study.

TABLE VI

VALENCE VIBRATIONS OF $-COO^-$ AND $-COOH(D)$ GROUPS

Substance	Solvent	Form	ν(sym.) $-C(=O)O^-$	ν(asym.) $-C(=O)O^-$	ν(CO) $-C(=O)OH(D)$
Glycine	H_2O	Anion	1400(10)	Masked by	.....
		Dipolar ion	1411(8b)	H_2O band	.....
		Cation	.....	.....	1743(6b)
	D_2O	Anion	1408(10)	1566(7b)	.....
		Dipolar ion	1409(8)	1623(3b)	.....
		Cation	.....	.....	1735(3b)
α-Alanine	H_2O	Anion	1413(8)	Masked by	.....
		Dipolar ion	1410(8)	H_2O band	.....
		Cation	.....	.....	1726(4b)
	D_2O	Anion	1408(8)	1563(2b)	.....
		Dipolar ion	1414(8)	1612(2)	.....
		Cation	.....	.....	1738(6b)
β-Alanine	H_2O	Anion	1406(10)		.....
		Dipolar ion	1405(9)	Masked	.....
		Cation	.....		1723(5b)
	D_2O	Anion	1406(10)	1560(2b)	.....
		Dipolar ion	1398(7)	1570(4b)	.....
		Cation	.....	.....	1707(6b)

all the amino acids and is essentially unaffected, in position or intensity, by ionization or deuteration of the amino group. In cationic α- and β-alanine a line is found in nearly the same position, but this is much less intense than the lines near 1400 in the dipolar ion or anion, and it obviously arises from a C–H deformation frequency which already has been discussed.

The asymmetrical stretching frequency of the ionized $-COO^-$ group is particularly easy to recognize in the deuterated amino acid solutions in D_2O, in which the ordinary water band near 1640 has been displaced to 1200 cm.$^{-1}$. All the anionic amino acids in D_2O show a moderately strong line near 1565; there is a similar line in the deuterated dipolar ions at a somewhat higher frequency—1623 in glycine, 1612 in α-alanine, but only 1570 in β-alanine. The identification of this frequency in the deuterated amino acids is particularly helpful, since any N–H deformation frequencies that may be present in this range in the ordinary amino acids would have been displaced to lower values.

Certain other frequencies may be associated with the ionized carboxyl group. There is a strong line near 1320 ± 10 in the anion and dipolar ion of glycine, in both D_2O and H_2O solution. There is a weaker line in the same range for the cation of glycine in H_2O, but not in D_2O. The dipolar ion of α-alanine has a very strong frequency at 1350 in H_2O, and at 1336 in D_2O, with weaker lines in the same range for the cation, and, also, in D_2O, for the anion. Also deuterated α-alanine shows a very strong line near 1290, present in the anion and dipolar ion, but not in the cation. In β-alanine, the line near 1370 is present in dipolar ion and anion, but absent or very weak in the cation. It is, however, very weak in the deuterated anion also, so that the interpretation remains uncertain. A line in the range 1350–1370 appears on ionization of the carboxyl groups of propionic acid, malonic acid, and the aminobutyric acids[4,5] but not in many other carboxylic acids.

There are some indications from other work that the deformation frequency of the ionized carboxyl group is near 600 cm.$^{-1}$, but we do not believe that any of the lines in our spectra can be definitely assigned to this frequency, except perhaps for the line near 660 in glycine and 640–650 in deuterated glycine.[16]

Some Skeletal Frequencies Involving C–C and C–N Linkages.—A few frequencies of this class are listed in the left-hand part of Table VII for glycine and β-alanine. It is probable that the motions involved are too complex to be described, even approximately, as simple C–C or C–N bond vibrations. More probably they involve the entire molecular skeleton, with modifications due to the attached carbon atoms. The frequency marked ν(C–C) in Table VII for glycine is the "sensitive frequency" of Edsall,[4] which decreases markedly when the carboxyl group attaches a proton and is also lowered by about 60 cm.$^{-1}$ in the anion or dipolar ion on deuteration of the amino or ammonium group. The downward displacement is nearly 100 cm.$^{-1}$, for the deuterated as compared with the undeuterated cation, *i.e.*, from 871 to 774, if indeed these two lines correspond to the same mode of vibration. Two or more strong frequencies are observed in all forms of β-alanine, in the region between 810 and 930; they are at somewhat lower frequencies in deuterated than in undeuterated β-alanine, but the shift is not large (see the discussion of β-alanine crystals below).

The "sensitive frequency" in α-alanine (Table II) is near 845, in the anion and dipolar ion, and at 819 in the cation. The lines at 806 in the deuterated dipolar ion and 786 in the deuterated cation may correspond to these; the corresponding frequency in the anion may be that at 823.

The assignment of the line near 500 in glycine to a bending motion of the C–C–O linkage is tentative, but seems probable in view of the work of Mizushima, *et al.*[16]

No attempt will be made here to assign the other frequencies in the reported spectra. We note that in general the spectra of the deuterated compounds appear somewhat more complex than those of the compounds containing ordinary hydrogen.

Studies on Amino Acid Crystals.—Raman spectra were determined for isoelectric glycine, α- and β-alanine in the crystalline state; in spite of the technical difficulties of such measurements, all the principal lines observed in the solutions of glycine and α-alanine were found in the crystal also. In the case of β-alanine, however, the spectrum of the solid gave lines as follows in the region 850–1200 cm.$^{-1}$ with a probable error near 10 cm.$^{-1}$: 850 (5), 885 (2), 940 (4), 1000 (4), 1060 (2), 1140 (1). The strong line at 970, observed in the solution, is missing or displaced; at any rate there are three strong lines between 900 and 1030 in the solution, and only two in the solid. It is probable that the line which has disappeared represents a skeletal mode corresponding to one of the different configurations of the $N–C–C–COO^-$ chain which can undergo interconversion through internal rotation about the bonds.[17] In the crystal one of

(16) H. Baba, H. Mukai, T. Shimanouti and S. Mizushima, *J. Chem. Soc. Japan*, **70**, 333 (1950). We are indebted to Prof. S. Mizushima for communicating to us the results of an infrared study on crystalline glycine which is to be published shortly in "Spectrochimica Acta."

(17) S. Mizushima, "Structure of Molecules and Internal Rotation," Academic Press, New York, N. Y., 1954.

TABLE VII[a]

SOME SKELETAL VIBRATIONS OF GLYCINE AND β-ALANINE

Substance	Solvent	Form	ν_{C-N}	ν_{C-C}	δ(<OCO)	δ(<CCO)
Glycine	H_2O	Anion	?	896(6)		512(3b)
		Dipolar ion	1031(3)	896(7)	667(1b)	511(3b)
		Cation	1043(4)	871(7)	657(vw)	503(2b)
	D_2O	Anion	?	834(2)?	650(vw)	482(vw)
		Dipolar ion	1000(2)	840(3)	640(1)	500(1b)
		Cation	1005(4)	774(4b)	640(vw)	490(vw)
β-Alanine	H_2O	Anion	1071(3b)?	876(4), 933(5)		
		Dipolar ion	1040(4)	866(8), 928(7)		
		Cation	1040(4)	817(7), 864(8), 906(3), 926(3)		
	D_2O	Anion	1033(1)?	900(5)		
		Dipolar ion	1023(3)	850(5), 890(6)		
		Cation	1019(4)	810(6), 845(5)		

[a] The frequencies labelled ν_{C-N} and ν_{C-C} are not to be ascribed uniquely to particular bonds; both probably involve the whole molecular skeleton. Detailed assignments cannot yet be made; see text.

these configurations is stabilized and the possibility of transition to the other is blocked.

Comparison with Infrared Data.—Our studies are closely related to the infrared measurements of Lenormant.[8b] His measurements in general give results quite concordant with ours. He has recorded a number of lines between 2600 and 2900 in the glycine spectrum which are not observed under our conditions in the Raman spectrum. These may be due to harmonics or combination frequencies. He reports a C–H stretching frequency at 2940 which is unchanged by deuteration, whereas in the Raman spectrum the C–H stretching lines of glycine occur at 2970 and 3010. The reason for this difference is not clear, but similar differences between infrared and Raman spectra are observed frequently in this region of the spectrum. The general agreement between Lenormant's measurements and ours may be regarded as very satisfactory.

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[CONTRIBUTION FROM THE BIOLOGICAL LABORATORIES, HARVARD UNIVERSITY]

Raman Spectra of Amino Acids and Related Compounds. X. The Raman Spectra of Certain Peptides and of Lysozyme[1-3]

BY DAVID GARFINKEL AND JOHN T. EDSALL[4]

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Raman spectra are reported for asparagine, glycylglycine, glycyl-DL-serine, glycyl-DL-valine, L-alanyl-L-alanine and D-alanyl-L-alanine. Glycylglycine was studied as the dipolar ion, anion and cation; the other compounds as the dipolar ion and cation only, except that asparagine was observed only as the cation in acid solution. Spectra of polylysine and of lysozyme from egg white are also reported. The carbonyl stretching frequency of the un-ionized –COOH group is weak or missing in the cationic forms of most of the peptides studied. The C–H stretching and deformation frequencies observed in the constituent amino acids are generally present without much change in the peptides, as are some other vibrations associated with the side chains. The skeletal frequencies characteristic of the free amino acids are missing or markedly displaced. The Raman spectra of LL- and DL-alanylalanine in solution are much more nearly alike than the infrared spectra of the same substances in the crystalline state, as determined by Ellenbogen. The Raman spectrum of lysozyme is faint, but some characteristic frequencies can be clearly identified. The spectrum of lysozyme is also faint, but reveals a number of frequencies not recorded in the infrared spectra reported earlier by other workers.

In the preceding papers[3] of this series the Raman spectra of free amino acids have been described. Peptides and proteins can also be studied by this technique, although the difficulty of recording the spectra photographically increases with increasing size of the molecule, since this increases the Rayleigh scattering, while the Raman scattering is roughly proportional to the mass of molecule per unit volume. This paper reports studies on several dipeptides, a polypeptide and a protein. Whenever it seems profitable, comparison is made with infrared spectra obtained by other workers on the same

(1) For the preceding paper of this series, see M. Takeda, *et al.*, THIS JOURNAL, **80**, 3813 (1958).

(2) Taken in part from the Ph.D. thesis of David Garfinkel, Graduate School of Arts and Sciences, Harvard University, 1955.

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