β_1-lipoproteins and the β_1-metal combining protein from the β_1-lipoproteins by this first precipitation of the sodium proteinates in 0.066 mole fraction ethanol at *p*H 5.8 at $-5°$. The γ-globulins may then be quantitatively separated from the precipitated sodium proteinates, and the serum albumins from the precipitated zinc proteinates, by fractional extraction in 0.051 mole fraction ethanol at *p*H 5.5 at $-5°$.

8. The sodium salts of most of these fractions are soluble in the ethanol–water systems from which the heavy metal–protein salts separate, and may often be crystallized. The heavy metal salts formed with most of these proteins are soluble in water. Removal of the metal from the protein by addition to the ethanol–water mixture of citrate, ethylenediamine tetraacetate, or a comparable reagent, redissolves the protein as the sodium salt. Passage of the water soluble metal salts through an appropriate exchange resin may also be employed to remove the metal ions.

9. Many protein–protein complexes that are insoluble under conditions such that one of the proteins alone is soluble—such as those between γ-globulins and β-lipoproteins—are decomposed by the addition of glycine to the system.

10. A new system, based upon protein–protein and protein–metal interactions, is proposed for the quantitative separation of the protein components of plasma or other tissues, for analytical or preparative procedures on any scale.

BOSTON 15, MASSACHUSETTS RECEIVED NOVEMBER 5, 1949

[CONTRIBUTION FROM THE DEPARTMENT OF PHYSICAL CHEMISTRY, HARVARD MEDICAL SCHOOL]

Raman Spectra of Amino Acids and Related Compounds. VII. Glycylglycine, Cysteine, Cystine and Other Amino Acids[1]

BY JOHN T. EDSALL,* JOHN W. OTVOS AND ALEXANDER RICH

This paper reports the continuation of earlier studies[2,3] on amino acids and related compounds. The spectrum of glycylglycine, the simplest of the peptides, shows certain features not observed in the amino acids. The presence in cysteine of the sulfhydryl group, with its characteristic frequencies sets it apart from the other amino acids. The study of cystine has also revealed the presence of the characteristic vibrational frequency of the S–S linkage. Spectra for the basic amino acids, lysine and histidine, and for some β-, δ- and ϵ-amino acids are also reported.

Experimental

The experimental technique has already been fully described.[2,3,4,5] All studies were made with the Hilger E-439 glass spectrograph. For the isolation of the mercury *e* line (22938 cm.$^{-1}$) a filter composed of an alcoholic solution of *p*-nitrotoluene and rhodamine 5 GDN extra was employed[6]; and for the isolation of the mercury *k* line (24705 cm.$^{-1}$) a combination of a dilute solution of sodium nitrite with Corning red purple ultra glass. The observed spectra are recorded in Table I, together with details concerning the materials and the preparation of the solutions studied. All the compounds reported, except cystine dihydrochloride, were studied using both the mercury *k* and *e* lines as the exciting radiation. Cystine dihydrochloride could be studied only with the *e* line, since the solution showed appreciable fluorescence when *k* line excitation was employed.

Discussion of Results

Cysteine and Cystine.—The most distinctive feature of the spectrum of cysteine is the stretching frequency of the S–H group at 2572 cm.$^{-1}$. This frequency for the cysteine cation in water is identical, within two or three cm.$^{-1}$, with the S–H frequency in liquid aliphatic mercaptans. Kohlrausch[7] gives for a series of 10 aliphatic mercaptans values of the S–H frequencies ranging from 2567 to 2573, with an average of 2571. If there were any hydrogen bonding between the sulfhydryl group and the surrounding water molecules, the corresponding frequency in cysteine should be decreased below its value in the mercaptans and the line should also be broadened. On the contrary, the frequency is unchanged and the line is sharp, indicating practically complete absence of hydrogen bonding.

The intense line at 684 cm.$^{-1}$ must represent the stretching frequency associated with the C–S bond, corresponding to the lines near 705 in

* Harvard University M.D. 1928; Chairman Board of Tutors in Biochemical Sciences, 1931–; Associate Professor of Biological Chemistry, 1938–.

(1) This work was supported by grants from the Rockefeller Foundation and from funds of Harvard University.

(2) J. T. Edsall and H. Scheinberg, *J. Chem. Physics*, **8**, 520 (1940).

(3) J. T. Edsall, THIS JOURNAL, **65**, 1767 (1943).

(4) (a) J. T. Edsall, *J. Chem. Phys.*, **4**, 1 (1936); (b) **5**, 225 (1937); (c) **5**, 508 (1937).

(5) J. T. Edsall and E. L. Sagall, THIS JOURNAL, **65**, 1312 (1943).

(6) J. T. Edsall and E. B. Wilson, Jr., *J. Chem. Phys.*, **6**, 124 (1938).

(7) K. W. F. Kohlrausch, "Ramanspektren," Hand- und Jahrbuch der chem. Physik," Band 9, Abschnitt VI, Leipzig, 1943 (lithoprint copy by Edwards Brothers, Ann Arbor, Michigan, 1945), see page 210.

TABLE I

EXPERIMENTAL DATA ON COMPOUNDS STUDIED

1. **Cysteine Hydrochloride** ($HS{\cdot}CH_2CH(COOH){\cdot}NH_3^+Cl^-$). The sample studied was a highly purified preparation obtained from Dr. J. P. Greenstein. It was dissolved in water at a concentration of approximately 2 *m* in the presence of an excess of hydrochloric acid, approximately *N* to repress ionization of the carboxyl group. The solution was filtered with Norit and promptly stoppered. The solution was extremely clear and free from dust particles: $\Delta\tilde{\nu}$: 526 (0b); 613 (0b); 684 (6); 781 (1); 799 (1); 866 (2); 938 (2); 998 (1); 1079 (1/2b); 1149 (0); 1220 (1); 1271 (0); 1324 (0); 1363 (0); 1428 (3); 1648 (1b); 1745 (3b); 2572 (6); 2956 (6); 3007 (2b); 3080 (1/2b). Preliminary polarization studies showed the lines 1745, 2572 and 2956 to be strongly polarized. A number of the other lines are probably polarized.

2. **Cystine Hydrochloride** ($[{-}S{\cdot}CH_2{\cdot}CH(COOH){\cdot}NH_3^+]_2[Cl^-]_2$). A commercial sample of L-cystine, which had been three times recrystallized, was dissolved in an aqueous solution in the presence of excess HCl at a *p*H of 0.6. The concentration of cystine was approximately 14% (0.65 *m*). The observed spectrum is certainly incomplete, due partly to the low concentration of cystine cation in the solution and partly to the presence of some fluorescence. The line at 2950 is certainly much more intense than the relative figure reported here, but it falls in a region of the photographic plate which is relatively insensitive. As stated above, it was impossible to obtain a usable photograph of cystine hydrochloride with the mercury *k* line as exciting line: $\Delta\tilde{\nu}$: 504 (6); 667 (6); 702 (1); 728 (1); 767 (1); 824 (1); 885 (1); 963 (1b); 1072 (1b); 1216 (1); 1424 (1); 1745 (1); 2950 (1vb).

3. **Glycylglycine** ($^+H_3N{\cdot}CH_2{\cdot}CONH{\cdot}CH_2{\cdot}COO^-$). Preparation obtained from Hoffmann-LaRoche. N calcd. 21.2, found 20.9. The solution studied was dissolved in water at 1.5 *m*. 0.03 *N* HCl was added to assist in clarification of the solution during filtration with Norit. The amount of glycylglycine cation so produced by the addition of acid was far too small to give rise to any detectable lines in the spectrum, as was shown by comparison with spectrum number 4 below: $\Delta\tilde{\nu}$: 227 (1/2b); 396 (2b); 530 (0b); 601 (0b); 727 (00); 884 (4); 922 (4); 1032 (2); 1135 (1vb); 1278 (5b); 1320 (2); 1395 (6); 1439 (2); 1618 (0vb); 1695 (1); 2945 (7); 2971 (7).

4. **Glycylglycine Hydrochloride** ($[^+H_3N{\cdot}CH_2{\cdot}CONH{\cdot}CH_2{\cdot}COOH][Cl^-]$). This was prepared from the glycylglycine used in spectrum number 3 above by addition of hydrochloric acid. On account of the limited solubility of the hydrochloride, it was not possible to obtain a greater concentration than approximately 0.9 *m*. An excess of 0.4 *N* HCl was added in order to repress ionization of the carboxyl group of glycylglycine, so that the observed measurements represent only the spectrum of the glycylglycine cation dissolved in water: $\Delta\tilde{\nu}$: 234 (1/2b); 404 (1); 882 (5); 1024 (1); 1146 (1/2); 1279 (4); 1416 (2); 1439 (2); 1637 (1); 1701 (1); 1740 (1); 2966 (7b).

5. **β-Alanine** ($^+H_3N{\cdot}CH_2{\cdot}CH_2{\cdot}COO^-$). Approximately 4 *m* solution in water. The solution gradually became cloudy during each run and had to be refiltered at the end of the run. It is probable that the observed spectrum is incomplete: $\Delta\tilde{\nu}$: 398 (1/2); 513 (1/2); 612 (1/2); 877 (3b); 934 (3); 979 (1/2); 1046 (2b); 1338 (1); 1410 (5b); 1471 (1/2); 2934 (6); 2991 (6).

6. **β-Alanine Hydrochloride** ($[^+H_3N{\cdot}CH_2{\cdot}CH_2{\cdot}COOH][Cl^-]$). Approximately 4 *m* solution containing approximately 0.4 *N* HCl to repress ionization of carboxyl group. There was considerable cloudiness in the solution which was largely, but not completely, removed after repeated filtration. The spectrum, however, appears more nearly complete than that of β-alanine itself: $\Delta\tilde{\nu}$: 385 (1b); 509 (0b); 821 (4); 865 (3); 914 (1/2); 945 (1/2); 973 (1); 1051 (3b); 1115 (1/2); 1261 (1/2b); 1328 (2b); 1413 (3b); 1472 (1b); 1629 (1b); 1730 (4b); 2942 (5); 2989 (5).

7. **β-Aminobutyric Acid** ($^+H_3N{\cdot}CH(CH_3){\cdot}CH_2{\cdot}COO^-$). Sample prepared in the laboratory of Prof. C. S. Marvel. Purified by three recrystallizations from alcohol-water; 2.7 *m* solution in water: $\Delta\tilde{\nu}$: 238 (0b); 354 0vb); 453 (0vb); 511 (0b); 837 (3vb); 913 (3b); 958 (1); 1005 (1b); 1123 (0); 1214 (0); 1277 (1/2b); 1366 (1/2); 1408 (7b); 1457 (3); 1631 (1vb); 2766 (1/2); 2914 (3); 2953 (5); 2996 (3).

8. **β-Aminobutyric Acid Hydrochloride** ($[^+H_3N{\cdot}CH(CH_3){\cdot}CH_2{\cdot}COOH][Cl^-]$). 2.7 *m* solution in water with slight excess of HCl: $\Delta\tilde{\nu}$: 240 (1/2b); 347 (1/2b); 457 (1/2b); 492 (1/2); 533 (1); 651 (?); 800 (1/2); 829 (4); 849 (0); 899 (5); 954 (1); 1004 (1b); 1108 (2b); 1145 (1/2); 1212 (1); 1264 (1); 1380 (2b); 1419 (2); 1461 (4); 1637 (2vb); 1726 (4vb); 2777 (2); 2900 (4); 2952 (6); 2996 (4).

9. **δ-Aminovaleric Acid Hydrochloride** ($[^+H_3N{\cdot}(CH_2)_4{\cdot}COOH][Cl^-]$). Prepared in the laboratory of Prof. C. S. Marvel. Recrystallized three times from alcohol-water. 3 *m* solution in water with slight excess of HCl: $\Delta\tilde{\nu}$: 225 (1/2b); 342 (1/2vb); 456 (1/2 vb); 815 (1); 867 (1/2); 903 (2); 1036 (2); 1072 (3); 1221 (1/2); 1325 (4b); 1452 (4b); 1632 (1b); 1723 (3b); 2888 (1); 2940 (6).

10. **ε-Aminocaproic Acid** ($^+H_3N(CH_2)_5{\cdot}COO^-$). This preparation was obtained from Dr. J. P. Greenstein and was a very carefully purified product. It was studied in water at a concentration of approximately 2.5 *m*: $\Delta\tilde{\nu}$: 501 (00); 637 (00); 851 (0); 892 (1); 941 (4); 1069 (3vb); 1153 (00); 1209 (0); 1320 (3); 1410 (4); 1450, (4); 2878 (2); 2927 (3).

11. **ε-Aminocaproic Acid Hydrochloride** ($[^+H_3N{\cdot}(CH_2)_5{\cdot}COOH][Cl^-]$). 2 *m* solution in water containing slight excess of HCl to repress ionization of carboxyl group. $\Delta\tilde{\nu}$: 492 (00); 617 (00); 847 (2); 929 (2); 1018 (1); 1068 (3b); 1208 (0); 1324 (3); 1449 (4); 1631 (1); 1725 (2); 2870 (2); 2925 (4).

12. ***dl*-Lysine Dihydrochloride** ($[^+H_3N{\cdot}(CH_2)_4{\cdot}CH(NH_3^+){\cdot}COOH][Cl^-]_2$). 2.5 *m* solution in water containing slight excess of HCl: $\Delta\tilde{\nu}$: 318 (0); 430 (0); 534 (0); 642 (0); 742 (1); 837 (1); 907 (1); 962 (0); 1013 (1); 1074 (2); 1142 (1); 1332 (4); 1448 (5); 1621 (2); 1741 (4); 2782 (0); 2875 (2); 2931 (4).

13. **Histidine Monochloride** ($[C_6H_4N_2^+{\cdot}CH_2{\cdot}CH(COO^-){\cdot}NH_3^+][Cl^-]$). Commercial sample, twice recrystallized from alcohol water as the monohydrate. Calcd. for $C_6H_{10}N_3O_2Cl{\cdot}H_2O$: Cl, 17.40; found 17.35. Solution in water, approximately 1.4 *m*: $\Delta\tilde{\nu}$: 538 (00); 644 (00); 715 (00); 825 (0); 867 (1); 946 (00); 1004 (2); 1095 (1); 1202 (4b); 1274 (4); 1358 (2b); 1437 (1); 1494 (4b); 1638 (3); 2961 (3); 3167 (4).

All these spectra, being taken in aqueous solutions, show the water bands at 3200–3600 cm.$^{-1}$. These are not listed in the data above; it must be noted that OH or NH stretching frequencies arising from the substance under study fall commonly in the region of the water bands, and will not be observed unless they are very strong. The characteristic stretching frequencies of the charged-NH_3^+ group fall commonly in the region below 3000 cm.$^{-1}$ and are obscured by the strong lines due to the C–H vibrations (see Edsall and Scheinberg[2]).

methyl mercaptan, 660 in ethyl mercaptan, and other lines or closely associated doublets in the dialkyl sulfides and dialkyl disulfides.[8] The corresponding frequency in the cystine cation is at 667. In addition, cystine shows an intense line at 504 which clearly corresponds to the vibration involving stretching of the S–S bond in the dialkyl

(8) R. Vogel-Högler, *Acta Physica Austriaca*, **1**, 311 (1948). The spectra reported by Vogel-Högler were observed on a spectrograph of high resolving power; hence many lines reported as single by most workers, including ourselves, were resolved into doublets. This should be borne in mind in evaluating the data in Table II.

disulfides.[8,9] Comparisons of certain values for these frequencies in cysteine, cystine and the dialkyl sulfides are given in Table II. The very weak line at 526 in the cysteine cation almost certainly represents a totally different vibration from the strong 504 line in the cystine cation (Table II).

TABLE II

S–H, C–S AND S–S FREQUENCIES

Compound	S–H	C–S	S–S	Reference
Methyl mercaptan	2574	698, 705	.....	a,b
Ethyl mercaptan	2571	658, 665	.....	a,b
n-Propyl mercaptan	2570	652, 695	.....	b,e
Cysteine (cation)	2572	684	.....	d
Dimethyl disulfide	..	686, 692	503, 508	a,c
Diethyl disulfide	..	640, 668	507, 523	a,c
Cystine (cation)	..	667	504	d

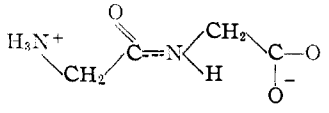
[a] R. Vogel-Högler, ref. 8. [b] Kohlrausch, ref. 7, p. 211. [c] Gerding and Westrik, ref. 9. [d] This paper. [e] Landolt-Börnstein "Physikalisch-Chemische Tabellen," Dritter Ergänzungsband, Zweiter Teil, Berlin, 1935, p. 1064.

Sheppard[10] has recently inferred that a deformation frequency of the S–H linkage in mercaptans has a characteristic value between 800 and 900 cm.$^{-1}$; in ethyl mercaptan its value is 838. On this numerical basis, the most probable assignment for this frequency in the cysteine cation would be the line at 866 which is moderately intense. However, this assignment must remain tentative.

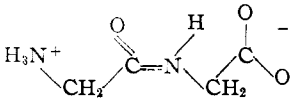
It is obvious that the frequency at 1745 in the cations of both cysteine and cystine is associated with the C=O vibration in the carboxyl group.[4] Although tentative identification of some of the other frequencies might be made, it seems better to defer their discussion until other related compounds have been studied.

Glycylglycine as Dipolar Ion and as Cation.—The vibration of the carboxyl group in glycylglycine produces marked changes in the spectrum. Several of these are already well-known from previous studies.[3] The disappearance of the C=O frequency at 1740 on ionization and its replacement by a lower frequency near 1400—in this case centered at 1395—is characteristic of the ionized carboxyl group. In glycylglycine the great intensity of the latter frequency in the dipolar ion is particularly striking. It is quite sharp and far more intense than the line at 1740 in the cation. The moderately strong line at 1320 in the dipolar ion is also absent in the cation.

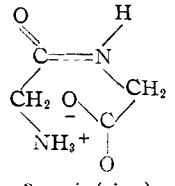
In the range near 1000 cm.$^{-1}$, where the stretching frequencies of the molecular chain appear, the dipolar ion contains two strong lines at 884 and 922; only the former is present in the cation. The appearance of the additional frequency at 922, when the carboxyl group acquires a negative charge, suggests that additional configurations of the chain may occur in the dipolar ion which are not found in the cation. No conclusive interpretation can yet be offered, but it should be noted that Hughes and Moore[11] have shown the C–N distance in the peptide linkage to be approximately 1.29 Å., indicating a high degree of resonance and of double bond character in the peptide linkage. Thus, the two CH_2 groups adjoining this linkage must be coplanar with the C, O and N atoms of the peptide group, and must have either the *cis* or the *trans* configuration relative to that group. Relatively free rotation should exist around the other C–C and C–N bonds. The work of Hughes and Moore shows clearly that the configuration in the crystal is the extended *trans* form; but in solution a certain amount of the *cis* form may exist also, either in the extended form (2), the ring form (3) or in some intermediate configuration. The electrostatic attraction between the charged ammonium and carboxyl groups in the dipolar ion would tend to favor the ring form, whereas the cation may exist in the presumably more stable *trans* form, since electrostatic attractions no longer tend to draw the ends of the chain together if the carboxyl group is not ionized. Even with retention of the *trans* configuration, considerable modification of space relations is possible by rotation of the terminal ammonium and carboxyl ion groups around the bonds joining them to the —CH_2— groups.



1. *trans* (extended)



2. *cis* (extended)



3. *cis* (ring)

The moderately strong line near 1700 is present in both the dipolar ion and the cation, and is unaffected by ionization. Presumably, therefore, it represents a stretching frequency characteristic of the peptide group. It would probably be an oversimplification to denote this as a C=O stretching frequency, since the C–N bond also possesses so much double bond character. The actual frequency may well represent a more complex type of motion involving both the C=O

(9) H. Gerding and K. Westrik, *Rec. trav. chim. Pays-Bas*, **61**, 412 (1942).

(10) N. Sheppard, *J. Chem. Phys.*, **17**, 79 (1949).

(11) E. W. Hughes and W. J. Moore, THIS JOURNAL, **71**, 2618 (1949).

and C–N linkages. It clearly corresponds to the frequency near 1650, found in the N-alkyl amides.[12] The upward shift in this frequency of 50 cm.$^{-1}$ in glycylglycine is presumably due to the polar character of the neighboring amino and carboxyl groups. It cannot be explained by the fact that the measurements on glycylglycine were made in aqueous solution, while the N-alkylamides were studied as pure anhydrous liquids; since we have found that the corresponding frequency near 1660 in acetamide is the same in aqueous solution as the values reported by other authors[13] for acetamide as a pure liquid or in the crystalline state. In addition to the intense frequency at 1700 there is also a weak frequency at 1637 in the cation, which becomes weaker and broader and is shifted to 1618 in the dipolar ion. Probably this frequency is not related to the peptide linkage, since frequencies of almost the same value appear in the spectra of many carboxyl and amino acids and their salts, even when there is no peptide group in the molecule.[4]

Other Amino Acids Studied and Their Salts.—The β-, γ- and ϵ-amino acids studied, as dipolar ions and as cations, show all the features previously discussed[4a,4c] in relation to the ionization of the carboxyl group, and further discussion of this point is not required here. It should be noted, however, that the C=O frequency for the un-ionized carboxyl group lies near 1740 or 1745 for α-amino acids, while it lies between 1720 and 1730 for the β-, γ- and ϵ-amino acids. For the unsubstituted fatty acids in water, the corresponding value is almost exactly 1720. Thus, the presence of an adjoining charged ammonium group in the α-position increases the frequency by 15–20 cm.$^{-1}$, but the effect becomes very small or negligible when the charged group is farther away. The lysine cation containing charged ammonium groups in both the α- and the ϵ-position shows the value of 1740 for this frequency, which is found in the other α-amino acids.

Some of the stretching frequencies of the molecular chain, between 800 and 1100 cm.$^{-1}$, are noticeably different in the dipolar ion and cation forms of some of the amino acids studied. However, there is no such striking change as that found in glycylglycine, and its seems premature at this time to attempt to interpret the data.

The reported spectrum for histidine represents only one state of the molecule—namely, the state of maximum total charge, with positive charges on the imidazole and the ammonium group and a negative charge on the carboxyl group. This spectrum invites comparison with that of imidazole itself and some of the substituted imidazoles,[14] but the available data indicate that the relations are complex and that it will be more profitable to defer discussion until other related compounds have been studied.

Summary

1. Raman spectra are reported for the cationic forms of cysteine and cystine, for glycylglycine, lysine, histidine and some β-, δ- and ϵ-amino acids. Most of the substances have been studied both as dipolar ions and as cations in aqueous solution.

2. The characteristic S–H, C–S and S–S vibrations have been identified in cysteine and cystine.

3. A brief discussion is given of certain features in the spectra of glycylglycine and the other amino acids studied.

(12) K. W. F. Kohlrausch and R. Seka, *Z. physik. Chem.*, **B43**, 355 (1939).

(13) Ref. 7, p. 275.

(14) K. W. F. Kohlrausch and R. Seka, *Ber.*, **71**, 985 (1938).

BOSTON, MASSACHUSETTS RECEIVED NOVEMBER 5, 1949

[CONTRIBUTION FROM THE FRICK CHEMICAL LABORATORY, PRINCETON UNIVERSITY]

The Dipole Moments and Structures of Some Alkyl Disulfides and of Methyl Trisulfide

BY LAWRENCE M. KUSHNER,[1] GEORGE GORIN[2] AND CHARLES P. SMYTH*

The chemical behavior and properties of organic trisulfides have been variously interpreted as indicating a straight (I) or a branched (II) chain structure. Recent evidence has almost uniformly favored the first formulation. From an electron diffraction study of methyl trisulfide Donohue and Schomaker[3] have, in fact, concluded that the molecule has structure I, and have given interatomic distances and angles. As a number of measurements had already been made in this Laboratory upon the dipole moments of di- and trisulfides,[4] it seemed of interest to correlate this conclusion with the results of these measurements. Since structure II might be expected to give rise to a comparatively high dipole moment because of the coördinate linkage, a study of the dipole moment of methyl trisulfide was undertaken as a means of distinguishing between the two struc-

I R—S—S—S—R II R—S—S(↑S)—R

* Harvard University Ph.D. 1921.

(1) Present address: National Bureau of Standards, Washington, D. C.

(2) Thiokol Corporation Fellow 1946–1948; present address: School of Chemistry, Rutgers University, New Brunswick, N. J.

(3) Donohue and Schomaker, *J. Chem. Phys.*, **16**, 92 (1948).

(4) Westlake, Laquer and Smyth, THIS JOURNAL, **72**, 486 (1950).