The latest paper on infrared spectra of peptides from the laboratories of Mizushima[5f] suggests that another band, at 1015 ± 15 cm.$^{-1}$, be considered as typical for the sequence...glycylglycyl ...in polypeptides or proteins. The argument is based on the observation that the 1015 band is present in peptides consisting only of glycine residues, as well as in polyglycine, and absent in poly-DL-alanine, poly-β-alanine, poly-glycyl-DL-phenylalanine, and the periodic polymer of (glycine-DL-alanine). From the spectra presented in this paper, one would have to draw the conclusion that these absences are fortuitous. It would seem that the explanation lies in the fact that Mizushima and his colleagues have prepared polymers composed of the racemic DL-amino acids. In tri- and tetraalanine bands at 1015 ± 15 cm.$^{-1}$ are observed in varying degrees of intensity. The band seems to be absent in trialanine (DLL) and tetraalanine (LDLL). This band is probably due to some type of skeletal vibration, since it shows up in varying degrees also in the spectra of polyfunctional peptides (paper III[4]), as well as in most of the racemic polyamino acids prepared by the Lossen rearrangement.[6] In conclusion, it should be pointed out that care must be exercised in applying information based on infrared spectra to the case of peptides in aqueous solution. It is quite possible that some frequencies are a function of the array of molecules in the solid state. Unfortunately, not enough material was available to undertake a study of the relation between infrared spectra and crystal lattice. It would also be interesting to apply spectra as a test for the existence of the helical configuration of peptides, since the tetraalanines should be able to form one hydrogen bond if they could fold into the 3.7 residue helix. There is no *a priori* reason to assume this should be the configuration for these relatively small molecules in the solid state, and none of the observed frequencies can so far be interpreted as representing such a folding.

(6) C. D. Hurd, L. Bauer and I. M. Klotz, THIS JOURNAL, **75**, 624 (1953).

PITTSBURGH, PENNSYLVANIA

[CONTRIBUTION FROM THE LABORATORIES OF THE DEPARTMENT OF BIOCHEMISTRY AND NUTRITION, GRADUATE SCHOOL OF PUBLIC HEALTH, UNIVERSITY OF PITTSBURGH]

Dissociation Constants of Peptides. III. The Effect of Optical Configuration on the Infrared Spectra of Polyfunctional Peptides[1a,b]

BY ERIC ELLENBOGEN[2]

RECEIVED JUNE 3, 1955

Infrared spectra were obtained on the following peptides: alanyllysylalanine (3L, LLD, LDL), alanyllysyldialanine (4L, LDLL), alanyllysyltrialanine (5L, LDLLL), α-alanylglutamic acid (LL, LD), α-glutamylalanine (LL,LD), α-glutamylglutamic acid (LL,LD), and γ-glutamylglutamic acid (LL,DD). Spectra were determined on mulls suspended in Nujol and fluorolube, and the range from 5000 to 625 cm.$^{-1}$ was scanned. Certain frequencies were tentatively assigned to specific vibrations. Changing the optical configuration of an amino acid residue caused marked changes in the spectra of those peptides when compared with the pure L-compounds. Some spectra were so "diffuse" that they resembled infrared spectra of protein.

In the preceding paper of this series[3] infrared spectra were reported on peptides of known configuration containing but a single amino group and a single carboxyl group. In this paper are reported infrared spectra of peptides containing two amino and two or three carboxyl groups. In each peptide the L-amino residue containing the additional functional group has been replaced by its D-isomer.

Experimental

Peptides.—H·Ala-Lys-Ala·OH (3L, LLD, LDL), H·Ala-Lys-Ala-Ala·OH (4L, LDLL), and H·Ala-Lys-Ala-Ala-Ala·OH (5L, LDLL) were part of the lots previously described.[4] The glutamyl peptides were obtained by the courtesy of Dr. H. A. Sachs.

Infrared Spectra.—For details of preparing the samples in nujol and fluorolube mulls, as well as for the spectra of the suspending media, see paper II of this series.[3] The spectra of the compounds are shown in Figs. 1 to 7.

Results

Tentative frequency assignments have been made for the compounds investigated,[5] and are listed in Table I.

Discussion

Frequency assignments were made on the basis of arguments similar to those already advanced.[3] The relative shifts of the intensities of the three bands tentatively assigned to CH and CH_2 rocking in alanyllysylalanine (3L, LLD, LDL) can again be due to mutual repulsions of the CH_3 and β-CH_2 groups of the side chains which are attached to the asymmetric carbon atom. The band near 2000 cm.$^{-1}$ is assigned to the NH vibration arising from the NH_3^+ group. It is present in all peptides containing lysine (monohydrochlorides) and is broadened in alanyllysyldialanine (4L) which was examined as the dihydrochloride. This particularly broad band might arise from the alpha and epsilon NH_3^+ groups whose frequencies could be so close together that they are not resolved by the particular instrument used.

Of great interest is the general nature of these spectra. On the whole, they begin to resemble spectra obtained on crystalline insulin.[6] In the tripeptides H·Ala-Lys·Ala·OH, distinct bands are

(1) (a) Presented in part at the Third International Congress of Biochemistry, 1955; (b) supported by a grant from the U. S. Public Health Service, National Institutes of Health.

(2) The author wishes to acknowledge his appreciation to Dr. F. A. Miller, Mellon Institute, Pittsburgh, Pennsylvania, for carrying out the infrared spectra determinations reported in this paper.

(3) E. Ellenbogen, THIS JOURNAL, **79**, 363 (1956).

(4) E. Ellenbogen, *ibid.*, **74**, 5198 (1952).

(5) Refs. 5a through 5f of (3).

(6) E. Ellenbogen, THIS JOURNAL, **77**, 6634 (1955).

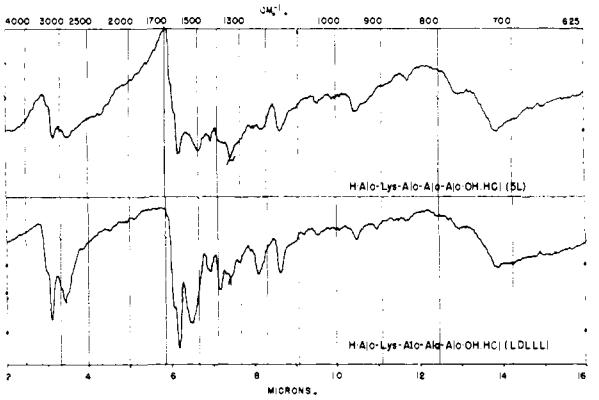

Fig. 1.

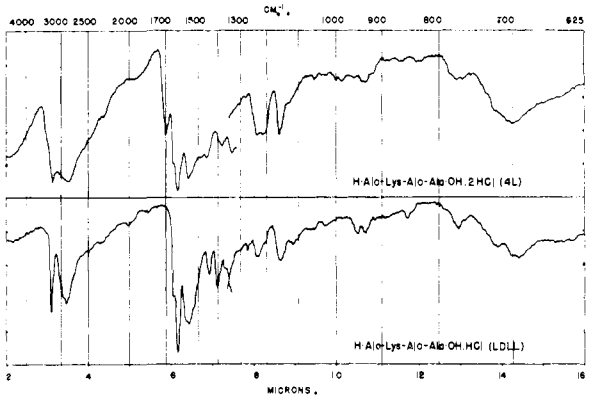

Fig. 2.

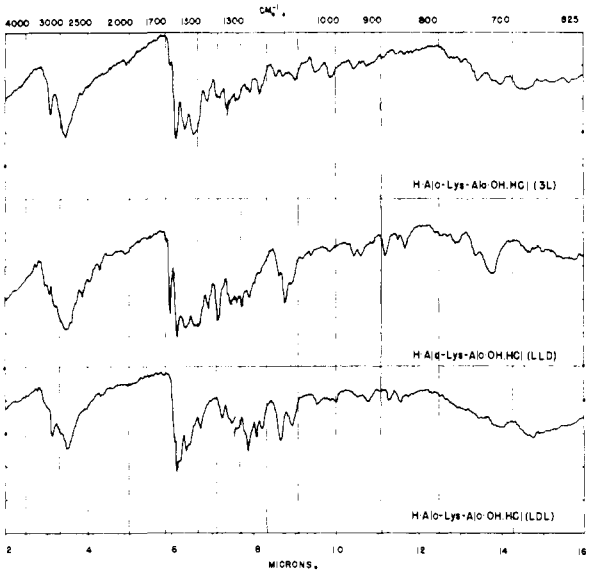

Fig. 3.

observed over the entire range scanned. Adding one and then two more alanine residues to the peptide, reduces the number of clearly recognizable bands very greatly. Comparing the spectra of these peptides with those of alanine peptides,[3] one would have to conclude that the presence of the second functional group gives rise to many more interactions in such a manner as to obscure many expected frequencies.

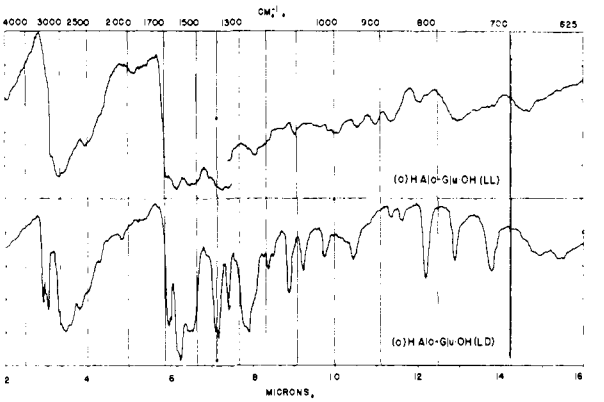

Fig. 4.

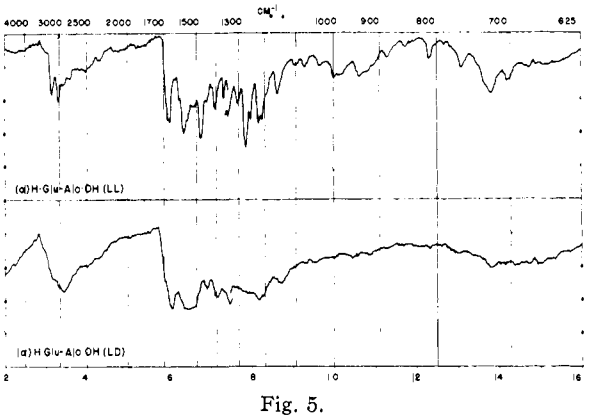

Fig. 5.

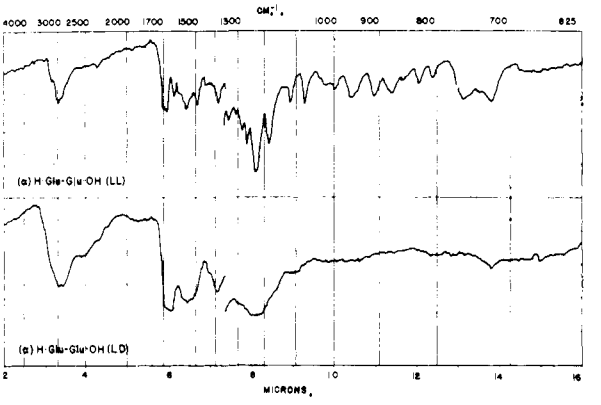

Fig. 6.

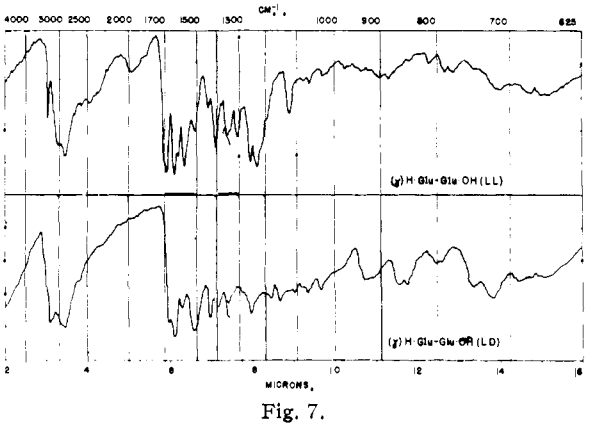

Fig. 7.

TABLE I

TENTATIVE FREQUENCY ASSIGNMENTS, CM.$^{-1}$

Assignment	H·Ala-Lys-Ala·OH ·HCl (3L)	·HCl (LLD)	·HCl (LDL)	H·Ala-Lys-Ala-Ala·OH ·2HCl (4L)	·HCl (LDLL)	H·Ala-Lys-Ala-Ala-Ala·OH ·HCl (5L)	·HCl (LDLLL)	α-H·Ala-Glu·OH (LL)	(LD)	α-H·Glu-Ala·OH (LL)	(LD)	α-Glu-Glu·OH (LL)	(LD)	γ-Glu-Glu·OH (LL)	(LD)
NH stretch H-bonded	3420	3420	3420	3330	3390	3300	3390	3410	3450	3195	3450	3330	3330	3420	3390
	3230	3275	3215	3190	3223	3210	3230	3300	3330	3010	3190	3175	2995	3280	3245
		3190	3030		3030	3030	3160	3210	3280		3030	3000		3180	3164
							3030	3030	3010					3030	3030
CH stretch	2910	2900	2890	2860	2950	2900	2940	2890	2900	2920	2900	2930	2900	2980	2990
		2800	2850		2870		2890			2790				2915	2890
NH stretch from NH_3^+	2040	2040	2020	1970	2020	1970	2060	1985	2070						
CO, CN stretch from peptide resonance	1630	1632	1630	1644	1645	1640	1645	1650	1628	1644	1640	1633	1640	1638	1640
	1572	1575	1608	1620	1600	1618	1618	1620	1600	1600	1574	1600	1575	1610	1587
			1570	1570	1573		1567	1575		1574		1568		1576	
CO stretch H bonded	1675	1700	1695	1700	1674	1694	1699	1695	1680	1700	1695	1725	1696	1700	1700
	1650	1674	1640					1665	1664	1670	1680	1700		1690	1676
										1660	1660	1675			
CN stretch and/or NH deformation	1528	1528	1550	1493	1553	1553	1550	1550	1538	1555	1540	1553	1550	1518	1525
	1504	1500	1520	1280	1528	1510	1540	1493	1490	1520	1493	1535	1470	1490	1508
	1270	1295	1490		1504	1278	1496	1266	1280	1505		1493	1260	1283	1284
	1262	1266	1266		1272	1258	1290		1264	1480		1475		1263	1257
		1250					1265			1270		1283			
										1252		1264			
CH deformation	1450	1450	1440	1453	1442	1440	1443	1440	1450	1450	1447	1450	1433	1450	1436
	1430	1404			1403				1410	1428	1440	1415	1400	1410	1398
	1412								1398	1407					
CNC stretch	1008	1010	1017	1000	1026	1010	1003	1004	1003	999		992		1015	785
	785	776		775	775	785	773	770	775	764		762		776	
CH, CH_2 rocking	957	957	952	955	952	955	955	947	957	940	955	957		940	925
	930	934	929	935	935	930	930	925	925	920	917	942			912
	913	916	908	916	917	915	914	917	918	913		913			
CH, NH rocking	725	722	717	700	720	720	730	730	735	730	733	722	723	718	720

This effect of a second and even a third functional group is well illustrated in the spectra of the glutamyl peptides. The bands of α-glutamylalanine (LL), α-glutamylglutamic acid (LL), γ-glutamylglutamic acid (LL), and, surprisingly, α-alanylglutamic acid (LD) are well resolved. One might infer that the mutual interaction between the functional groups in these four peptides is essentially confined to the formation of *inter*molecular hydrogen bonds. The spectra of their diastereoisomers (LD-compounds), on the other hand, are quite "diffuse," and this may be indicative of strong *intra*molecular interactions in addition to the *inter*molecular ones. The possibility of different types of mutual interactions can be qualitatively confirmed by constructing models of the four glutamylglutamic acid molecules. In the LD-isomer of the α-peptide, the two γ-carboxyl groups are free to interact with each other, as well as to bend in such a manner that one interacts with the amino group and the other with the terminal α-carboxyl group. The latter mode of interaction is somewhat more plausible, since the mutual repulsion of the side chain CH_2 groups (which leave the peptide linkage essentially at the same side) would tend to spread them apart. A strong interaction between the γ-carboxyl group and the terminal amino group could also explain the diffuse spectrum of α-glutamylalanine (LD). Models of γ-glutamylglutamic acid (LL,LD) do not shed much light on this problem, however. These models show that rotation about the amino terminal asymmetric carbon remains free, so that pronounced *intra*molecular interactions need not be postulated. They do take place, however, to some extent, as evidenced by the spectra of these two diastereoisomers, especially between 3400 and 3000 cm.$^{-1}$. These frequencies are shifted toward the lower end when going from the LL to the LD compound. In addition, the band near 925 cm.$^{-1}$ is quite pronounced in the LD and rather weak in the LL isomer. An increase in the strength of *intra*molecular hydrogen bonds in LD might explain this phenomenon.

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