[CONTRIBUTION FROM THE LABORATORY OF BIOCHEMISTRY, NATIONAL CANCER INSTITUTE, NATIONAL INSTITUTES OF HEALTH]

Studies on Diastereoisomeric α-Amino Acids and Corresponding α-Hydroxy Acids. V. Infrared Spectra

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The infrared absorption spectra in the solid state have been observed for 53 pairs of optically enantiomorphic α-amino acids, including five diasymmetric amino acids, in the wave length range 2 to 15 μ. No differences were noted in the spectra of the corresponding L- and D-forms of these compounds, but differences in varying degrees were noted between the diastereoisomeric forms of each of the amino acids with two centers of optical asymmetry. Most of the amino acids possessed a remarkable similarity in the 6.3 to 7.5 μ region of the spectrum, with a somewhat lesser uniformity in the 3.3, 3.9 and 4.7 μ regions. Using the spectra of the group of homologous straight chain α-amino acids as a prototype, it was possible in some cases to follow the effect of substituents on this chain by progressive shift in absorption bands in the 6 μ region.

Introduction

The infrared absorption spectra of α-amino acids have been the subject of a large number of investigations.[1] Most of these studies were conducted on amino acids in the solid state, although Gore, Barnes and Peterson[2] have demonstrated the usefulness of aqueous phase infrared methods, and Lacher, Croy, Kianpour and Park[3] have suggested the use of antimony trichloride as a solvent for obtaining the infrared absorption spectra of amino acids. Of considerable value in the interpretation of the ionic state of the amino acids, and of the assignment of characteristic frequencies to the $-NH_3^+$ and $-COO^-$ groups involved, has been the work of Edsall on the Raman spectra of the amino acids and related compounds in aqueous solution.[4] As a result of these various spectroscopic studies it may be accepted (a) that the α-amino acids in the solid state or at their isoelectric points in aqueous solution exist almost exclusively as dipolar ions, and (b) as a consequence of this dipolar ion structure many amino acids possess a characteristic absorption frequency at about 6.3 μ which is related to the $-COO^-$ group, as well as a relatively weak absorption at about 4.7 μ which may be attributed to NH frequencies in the $-NH_3^+$ ion.[5]

Beyond these basic assignments it has been difficult to interpret further the seemingly complex infrared spectra of the amino acids. This may be due in part to lack of systemization in the choice of the compounds used for spectral studies. In many cases the groups of amino acids selected have included those of the L- as well as those of the DL-configuration, although there is considerable evidence at hand that the infrared spectra in the solid state of an amino acid in these two optical forms are not comparable.[6-8] A further puzzling observation is the report by Gore and Petersen that the spectra of L- and D-threonine in the solid state exhibit distinct differences.[9]

The availability in this Laboratory of some 50 pairs of optically enantiomorphic α-amino acids, characterized analytically, optically and enzymatically, each isomer containing less than 0.1% of its antipode,[10-12] has provided the opportunity of making a systematic survey of their solid state absorption spectra for the following purposes, (a) to ascertain those bands in the 2 to 8 μ region common to this large series of amino acids and to observe the effects of substituents in these compounds, (b) to determine over the 2 to 15 μ region whether optically enantiomorphic pairs of amino acids do or do not exhibit identical spectra, and (c) to determine the extent of differences in the spectra from 2 to 15 μ of such diastereomeric pairs of amino acids as threonine–allothreonine, isoleucine–alloisoleucine, phenylserine–allophenylserine, hydroxyproline–allohydroxyproline, and α-aminotricarballylic acid–allo-α-aminotricarballylic acid, as well as the pyrrolidone and lactone forms derived from the last-mentioned amino acid.

Experimental

The spectra were taken on a model No. 21 Perkin–Elmer spectrophotometer equipped with sodium chloride optics. All samples were dried over P_2O_5 *in vacuo* before being pressed into KBr windows. The procedure for preparing these windows was essentially similar to that of Stimson and O'Donnell[13] and Scheidt and Reinwein.[14] An approximately 1% mixture of the compound with potassium bromide (Merck, Reagent Grade) was ground to pass a 200 mesh screen; 100 mg. of this mixture was transferred to an evacuable die having a diameter of 3/8" and after three to four minutes of evacuation pressed for two minutes at 14,000 pounds per square inch. The window so obtained was

(1) The literature up to 1952 has been considered by G. B. B. M. Sutherland in *Advances in Protein Chem.*, **7**, 291 (1952). References to later work in this field are to be found in the annual surveys of infrared spectra by Gore: *cf.* R. C. Gore, *Anal. Chem.*, **26**, 11 (1954).

(2) R. C. Gore, R. B. Barnes and E. Petersen, *ibid.*, **21**, 382 (1949).

(3) J. R. Lacher, V. D. Croy, A. Kianpour and J. D. Park, *J. Phys. Chem.*, **58**, 206 (1954).

(4) J. T. Edsall, *Cold Spring Harbor Symp. Quant. Biol.*, **6**, 40 (1938).

(5) H. W. Thompson, D. L. Nicholson and L. N. Short, *Faraday Soc. Disc.*, No. 9, 222 (1950). *Cf.* I. M. Klotz and D. M. Gruen, *J. Phys. Colloid Chem.*, **52**, 961 (1948).

(6) N. Wright, *J. Biol. Chem.*, **120**, 641 (1937); **127**, 137 (1939).

(7) S. E. Darman, G. B. B. M. Sutherland and G. R. Tristram, *Biochem. J.*, **42**, 508 (1948).

(8) Other examples of spectral differences of optical isomers and racemates in the solid state occur for the tartrates; *cf.* C. Duval and J. Lecomte, *J. Chem. Phys.*, **18**, 117 (1950), for substituted dicarboxylic acids; *cf.* A. Rosenberg and L. Schotte, *Acta Chem. Scand.*, **8**, 867 (1951), and for the acid phthallic ester of *p*-ethylphenylmethylcarbinol, *cf.* E. L. Eliel and J. T. Kofron, THIS JOURNAL, **75**, 4585 (1953). In general, the spectra of corresponding optically active and racemic forms do not differ for solutions of these compounds.

(9) R. C. Gore and E. M. Petersen, *Ann. N. Y. Acad. Sci.*, **51**, 924 (1949).

(10) J. P. Greenstein, S. M. Birnbaum and M. C. Otey, *J. Biol. Chem.*, **204**, 307 (1953); J. P. Greenstein, *Advances in Prot. Chem.*, **9**, 121 (1954).

(11) M. C. Otey, J. P. Greenstein, M. Winitz and S. M. Birnbaum, THIS JOURNAL, **77**, 3112 (1955).

(12) A. Meister, L. Levintow, R. B. Kingsley and J. P. Greenstein, *J. Biol. Chem.*, **192**, 535 (1951).

(13) M. M. Stimson and M. J. O'Donnell, THIS JOURNAL, **74**, 1805 (1952).

(14) U. Scheidt and H. Reinwein, *Naturforsch.*, **7b**, 270 (1952); *cf.* J. L. Hales and W. Kynaston, *The Analyst*, **79**, 702 (1954).

placed in the sample beam, and a similar window lacking the compound placed in the reference beam. In addition to these measurements made in the solid state of all the compounds, a few were made of selected compounds in D_2O solution, *cf.* ref. 2.

The α-amino acid L- and D-isomers were all prepared in this Laboratory, for the most part by the enzymatic resolution procedure described elsewhere.[10,11,15] Each was crystallized several times, was found to be analytically pure, and when tested with the appropriate amino acid oxidase or decarboxylase was determined to contain less than 0.1% of the optical antipode. A further test of their chemical purity has been noted recently in this Laboratory by the lack of toxicity of these amino acids when administered intravenously in relatively large amounts into experimental animals[16] and into a human being.[17] There has been no pharmacologic evidence at any time for the presence of pyrogens in these compounds. All were pure white in color.

Discussion

The solid state infrared spectra of the individual L- and D-isomers of some 50 α-amino acids and their derivatives have been examined between 2 and 15 μ. It may be stated at the very outset that in every case the spectrum of an L-amino acid was indistinguishable over this wave length range from that of its D-antipode. This finding not only disposes of one of the objectives of the present study, but provides a check both of the reliability of the measurements and of the chemical and optical purity of the compounds. Table I lists and characterizes the spectral absorption bands in the 2 to 8 μ region which is that in which the specific vibrational frequencies of the α-amino acids as a class would be expected to appear. In this table, the compounds are categorized into eight general groups based essentially upon obvious structural considerations.

The general structure of the α-amino acids may be represented by $RCH(NH_3^+)COO^-$. Group I compounds in Table I constitute a homologous series of straight chain α-amino acids which as a whole constitute a prototype of this class of compounds. An analogous homologous straight chain series in which an ω–OH group, and ω–COOH group, and an ω–NH_2 group are substituted, form the substance, respectively, of groups IV, VI and VII. Group II is composed of compounds in which R consists of various branched hydrocarbon chains, and group III is composed of compounds in which aromatic or corresponding alicyclic rings are substituted on the β-carbon atom of the α-amino acid. Group V consists of the alicyclic α-amino acids, and group VIII consists of a miscellaneous group of α-amino acids all of which contain sulfur.

Inspection of Table I reveals first of all a marked similarity in the infrared spectra of many of the amino acids in the region of 6.3 to 7.5 μ. Within this range six absorption peaks appear to be shared in common with most of the compounds studied, namely, 6.3, 6.6, 6.9, 7.1, 7.3 and 7.5 μ. The bands at 6.3 and 7.1 μ have been related, respectively, to the antisymmetrical and symmetrical stretching vibrations of the ionized carboxyl group of the dipolar ions,[2,18,19] 6.6 to an N–H deformation motion of the α-amino group,[3,20–23] the 6.9 and 7.3 μ bands are, respectively, related to antisymmetrical and symmetrical CH_3 stretching and possibly CH_2 deformation motions, and finally the 7.5 μ band may be considered to be related to a CH_2 wagging motion.[18,24]

The apparent exceptions to this uniformity in spectra include threonine, proline, allohydroxyproline and alloaminotricarballylic acid in which the 6.3 μ band is not observed; isovaline, leucine, serine, homoserine, hydroxyproline and allohydroxyproline, in which the 6.6 μ band is not observed; and valine, phenylalanine, allothreonine, hydroxyproline, aspartic acid, glutamic acid, aminoadipic acid, alloaminotricarballylic acid, α,β-diaminopropionic acid, ornithine and lysine, in whose spectra the 6.9 μ band is not apparent. None of the amino acids studied lack the 7.1 μ band. In the spectrum of glycine, however, the 7.3 μ band, and in that of alanine, the 7.5 μ band, is not apparent. Other absorption bands shared by many but not all of the amino acids are the 3.3 and 3.9 μ peaks which are provisionally assigned to the N–H and C–H stretching motions, and the 4.7 μ peak which is tentatively assigned to an N–H stretching vibration in the $-NH_3^+$ group of the dipolar ion form of the amino acid.[25] Perhaps one of the most striking differences between group I amino acids and most of those in other groups is the nearly complete absence of the 6.2 μ band in the former.

Compounds in Group I.—This homologous series of straight chain α-amino-α-carboxylic acids all possess five absorption peaks in common, namely, at 4.7, 6.3, 6.6, 6.9 and 7.1μ. All but aminocaprylic acid possess a band at 3.9 μ, all but glycine possess a band at 7.3 μ, and all but alanine possess a band at 7.5 μ. The lack of the 7.3 μ peak in the spectra at glycine may be due to the shortness of the skeletal chain and the absence of a free methyl group. In the case of alanine, the N–H deformation at 6.6 μ appears to be very weak, the antisymmetrical carboxylate vibration is split into two bands, absorbing at 6.15 and 6.25 μ, while the absorbance of the CH_3 bending motion is the most marked of any compound in this group. As the number of methylene groups in the chain increases, the absorbance at 7.3 μ tends to diminish. The hydrogenic N–H stretching motion at 4.7 μ is relatively weak for all the compounds of this group. Of all the amino acids in group I, only glycine possesses a band below 3.3 μ, namely, at 3.1 μ. However, the latter band occurs in the spectra of amino acids in other groups. The apparent lack of regularity in the bands in the 2.0 to 3.9 μ region may be a reflection of the strong hydrogen bonding of amino acids in the solid state,[1] and the consequent perturbation of the O–H and N–H stretching frequencies. The resulting shift in many instances toward absorbance

(15) S. M. Birnbaum, L. Levintow, R. B. Kingsley and J. P. Greenstein, *J. Biol. Chem.*, **194**, 455 (1952).

(16) Observed by Dr. P. Gullino.

(17) Observed by Dr. John Fahey.

(18) L. H. Jones and E. McLaren, *J. Chem. Phys.*, **22**, 1796 (1954).

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(21) H. Letaw, Jr., and A. H. Gropp, *J. Chem. Phys.*, **21**, 1621 (1953).

(22) R. D. B. Fraser and W. C. Price, *Nature*, **170**, 490 (1952).

(23) Note objections to this assignment by H. Lenormant, *Faraday Soc. Disc.*, No. 9, 319 (1950).

(24) T. Nakagawa and S. Mizushima, *J. Chem. Phys.*, **21**, 2195 (1953).

(25) L. A. Duncanson, *J. Chem. Soc.*, 1753 (1953).

TABLE

ABSORPTION SPECTRA IN THE INFRARED IN

	2 – 5μ							
I								
Glycine	3.17MS	3.45sh	3.50MB	3.73sh	3.90WS	4.75WB		
Alanine		3.30SS		3.73SB	3.90MS	4.77WB		
α-Aminobutyric acid			3.50SB		3.85sh	4.73WB		
Norvaline		3.40SB			3.87sh	4.73WB		
Norleucine		3.37SB		3.65sh	3.90sh	4.71WB		
α-Aminoheptylic acid		3.40SB			3.90sh	4.73WB		
α-Aminocaprylic acid		3.36MS		3.68MB		4.73WB		
α-Aminononylic acid		3.43SS	3.52sh		3.90sh	4.73WB		
α-Aminodecylic acid			3.57SB		3.90sh	4.75WB		
α-Aminoundecylic acid			3.57SB		3.90sh	4.73WB		
α-Aminododecylic acid			3.57SB		3.90sh	4.73WB		
II								
Valine		3.38SB		3.75SB	3.92sh	4.79MS		
Isovaline	2.97SS [3.18sh]	3.32SB		3.80SB	4.02SB	4.97WB		
Leucine		3.39SB		3.75MB	3.93MB	4.75WB		
Isoleucine		3.44SB			3.90sh	4.77WB		
Alloisoleucine		3.44SB			3.90WS	4.78WB		
t-Leucine			3.48SB		3.88WS	4.87WB		
III								
α-Aminocyclohexylacetic acid		3.37SB				4.77WB		
α-Aminocyclohexylpropionic acid	2.98MS	3.27SB	3.60sh		3.92sh	4.77WB		
α-Aminophenylacetic acid			3.47SB		3.90vw	4.82WB		
Phenylalanine		3.33SS				4.90MB		
Tyrosine	3.16sh	3.25SB	3.38sh	3.75SB	3.90sh	4.85WB		
Tryptophan	2.96MS	3.34MS			4.00MB	4.85WB		5.98MS
IV								
Serine	2.95MS	3.35SB		3.80WB	3.90sh	4.92WB		
Homoserine	3.15MS	3.40SS		3.70WB	3.90sh	4.80WB		
Homoserine lactone·HCl		3.40SB	3.52sh				5.10WB	5.63SS
Pentahomoserine	3.05sh [3.15sh]	3.37SB		3.63sh		4.73WB		
Hexahomoserine	3.06MS	3.40MB		3.66MB		4.73WB		
Phenylserine	2.83MS [3.15SB]	3.33sh			3.95WB		5.13WB	
Allophenylserine	3.00sh	3.30SB					5.00WB	
Threonine	3.20SB	3.40SB				4.90WB		
Allothreonine		3.30SB				4.90WS		
V								
Proline		3.33MB [3.38sh]		3.65vw				
Hydroxyproline	3.10SS	3.23MS	3.47MB	3.76MB	3.93MB [4.12vw]			
Allohydroxyproline	3.17SS		3.45MB		3.90MB			

I

THE 2 TO 8 μ REGION[a,b,c]

5 – 7μ						7 – 8μ			
	6.23SB		6.60SB	6.93WS	7.08SS		7.50SS		
6.15SS	6.28SS		6.65WB	6.88MS	7.08SS	7.35SS		7.65SS	
	6.33SB		6.63SS	6.90WS [6.85MS]	7.10SS	7.37MS	7.55SS	7.67WS	
	6.33SB		6.63SS	6.92WS	7.10SS	7.42MS	7.57WS	7.74WS	
	6.32SB		6.62MB	6.92WS	7.10SS	7.39MS	7.55SS	7.83WS	
	6.33SS		6.63MS	6.93MS	7.10SS	7.40WS	7.57MS		7.93vw
	6.33SS		6.62SS	6.93WS	7.10SS	7.38WS	7.58MS		8.02vw
	6.33SS		6.62MS	6.93WS	7.10MS	7.38WB	7.57MS		
	6.34SS		6.62SS	6.94MS [6.84vw]	7.13SS	7.39MS	7.58SS		
	6.34SS		6.64SS	6.94MS	7.12SS	7.37MB	7.57SS		
	6.35SS		6.63SS	6.94MS	7.12SS	7.37MB	7.57SS		
6.20sh	6.35SS		6.67SS		7.02WS [7.18MS]	7.40WS	7.53SS	7.87WS	
6.09sh	6.35SB			6.95MB [6.85sh]	7.11SB	7.28MS	7.45MS	7.75SS	
6.20sh	6.34SB		6.62MS	6.95WS	7.10SS	7.35MB	7.45sh	7.61MS	7.72MS
6.20sh	6.32SB		6.60SS	6.84WS	7.06MS [7.16SS]	7.40MS	7.52MS	7.64WS	7.86vw [7.96vw]
6.20sh	6.32SB		6.65SS	6.85WS	7.03MS [7.18SS]	7.39MS	7.60SS	7.80vw	7.96vw
	6.26SB	6.45sh [6.53sh]	6.76MB	6.83sh	7.12SS [7.18sh]	7.30WS	7.45SS	7.73SS	7.98vw
6.22sh	6.33SS		6.65SS	6.93vw	7.07MS [7.20MS]	7.42MS	7.50vw	7.62MS	7.87vw
	6.33SB		6.66MS	6.94WS	7.10SS	7.30WB [7.37sh]	7.50MS [7.67WS]	7.76vw	7.98vw
6.23SS	6.32sh	6.45sh	6.63SS	6.90vw	7.14MS	7.40WS		7.70vw	7.89vw
6.17MS	6.30SS		6.66SS		7.10SS		7.50WS	7.65MS	7.73sh
6.22SS	6.30SS		6.62MS	6.95vw	7.06MS	7.34MS	7.50SS		7.90sh [8.03SS]
	6.28SS			6.90vw	7.07SS	7.36SS	7.60vw		7.12vw
	6.25SB			6.81MS	7.08MS	7.22sh	7.46MS	7.67MS	
6.21sh	6.34SB	6.50sh		6.82vw	7.10SS	7.26WS	7.46SS	7.72MS	8.00MS
	6.28WB		6.66MS		7.18MS	7.33MS		7.67WS	8.25SS
	6.35SS		6.57SS	6.94WS	7.12SS	7.40MS	7.55MS		7.86WS
	6.36SS		6.65SS	6.96MS	7.14SS	7.42MS	7.59MS		8.05WB
6.00SS	6.25SB [6.32sh]	6.52sh	6.62SB [6.67sh]	6.90MS	7.18SS	7.40SS			
6.20SB	6.32sh	6.52sh	6.70WS	6.90WS	7.11SS	7.40WS		7.65SS	7.85WS
6.14SS			6.77SS	6.87SS	7.05SS	7.43SS [7.28sh]	7.60WS		8.00MS
6.10SS	6.27vw		6.80SS		7.07MS	7.35WS	7.50WS	7.75WS	7.93WS
6.18SS	6.43SS	6.55sh		6.92MS	7.13sh	7.29SB	7.57WS	7.75MS	7.98WS
6.10MS	6.31SB				7.01WS [7.15MS]	7.37WS	7.58MS	7.80WS	7.96MS
6.13SS		6.42MS		6.97MS	7.05sh	7.23MS	7.53MS	7.63vw [7.70vw]	7.93MS

TABLE

	2 –5μ						
VI							
Aspartic acid		3.34sh	3.43SB		4.85WB	5.25WB	5.93SS
Glutamic acid		3.32SS		3.70sh			
α-Amino-adipic acid		3.37SB		3.70vw	4.00vw	5.20MB	
Succinic acid		3.40SB		3.85sh	3.98sh		5.80SS
							5.92SS
							5.72sh }
Tricarballylic acid		3.27SB					5.83SB }
							5.90sh }
Allo-α-aminotricarballylic acid		3.26SB	3.44vw	3.85vw		5.25WB	5.80 } SB
							5.92 }
α-Aminotricarballylic acid	3.09SS	3.27SB	3.42sh				5.76SB
Allopyrrolidonedicarboxylic acid	2.87MS [3.14WS]		3.50MB		4.00vw		5.80SS
Pyrrolidonedicarboxylic acid	3.15MS				4.02vw	5.75SS	5.86MS
Isocitric acid lactone	3.00SB					5.60SS	5.80SS
Alloisocitric acid lactone	3.00SB					5.63SS	5.83SS
VII							
α,β-Diaminopropionic acid·HCl		3.30SB				5.08WB	
α,γ-Diaminobutyric acid·HCl	3.20SS	3.40SB			4.00WS	4.97WB	
Ornithine·2HCl		3.30SB					5.73SS
Lysine·HCl			3.50SB			4.80WB	
Arginine·HCl	3.07SS	3.27SS	3.50SB			4.77WB	5.95SS
Citrulline	3.05SS	3.17sh	3.34SB	3.45sh	3.90sh	4.78WB	5.95SS
Histidine		3.40SB		3.62sh			
VIII							
Methionine			3.50SB		3.95MB	4.76MB	
Ethionine		3.40SB		3.75sh	3.90MB	4.75WB	
Cystine			3.47SB		3.90sh	4.80WB	
Homocystine		3.50SB	3.57sh	3.75sh	3.90sh	4.75WB	
Cysteine·HCl			3.46SB		3.90sh	4.80WB	
S-Benzylcysteine	3.20sh		3.55SB		3.90MB	4.94WB	
S-Benzylhomocysteine	3.18sh		3.50SB	3.70sh	3.90MB	4.80WB	

[a] Succinic and tricarballylic acids have been included for comparison. [b] The bracketed numbers are so placed because of spatial limitations and are not to be regarded as special designations. [c] W = weak intensity, S = sharp band, sh = shoulder, M = medium intensity, B = broad band, vw = very weak band, S = strong intensity.

at longer wave lengths makes it very difficult to differentiate between the O–H, N–H and C–H stretching motions.

Compounds in Group II.—The branched-chain, monoaminomonocarboxylic acids, like those in group I, possess the 4.7, 6.3 and 7.1 μ peaks in common. In addition, they all possess the 3.9, 7.3 and 7.5 μ peaks in common, as well as, with the exception of *t*-leucine, the 3.3 and 6.2 μ bands and with the exception of isovaline, the 6.6 μ band. The amino acids in this category possess a branched chain residue on the α-carbon atom (isovaline), on the β-carbon atom (valine, isoleucine, alloisoleucine, *t*-leucine), or on the γ-carbon atom (leucine). Of all these compounds, the spectrum of leucine most closely resembles that of the amino acids in group I, a reflection no doubt of the relative distance along the chain of the branched methyl group from the amino and carboxyl radicals. *t*-Leucine possesses three methyl groups on the β-carbon atom, and this crowding apparently produces a marked interaction with the N–H banding motions of the α-amino group so that the characteristic frequency is no longer resolved as a single absorption band but is shifted into the region of carboxylate ion absorption to produce a strong, broad, poorly resolved band. The spectrum of *t*-leucine shows other characteristics which may be related to the unusual structure of this compound; thus a splitting of the symmetrical stretching frequency of the carboxylate ion seems to occur, producing two absorption bands at 7.12 and 7.18 μ. The symmetrical CH_3 stretching frequency appears at 7.29 μ, the C–H twisting motion is very strong and sharp at 7.45 μ, and the C–H deformation or CH_3 antisymmetrical stretching frequency splits into two poorly resolved bands

I (*Continued*)

5 − 7μ					7 − 8μ				
6.06MS	6.25MB		6.65SS		7.03SS	7.42MS	7.57SB		8.01SS
6.07SS	6.30vw		6.60SS		7.04MB	7.38SS		7.62MS	7.94SS
6.00SS	6.33SB		6.67SB		7.04MB	7.33MS	7.55SS	7.73SS	8.00MS
[6.07SS]						[7.40WS]			
					7.06SS			7.66SS	
				6.98SS		7.47WS		7.73MS	8.05SS
6.17WS		6.47vw	6.55MB		7.04sh	7.37MS	7.54MS	7.80SB	
					[7.12SB]				
6.16WS	6.30MS		6.63SS	6.90vw	7.10SB	7.40vw		7.66MB	7.80vw
6.09SS						7.25MS	7.57WS		8.00SS
6.02SS				6.94MS	7.12MS	7.32MS	7.45MS	7.62WS	7.87vw
								[7.78vw]	[8.13SS]
6.12sh					7.07MB		7.50WS	7.67WS	8.00MS
6.12vw					7.05MB	7.36WS	7.47WS	7.80MS	
	6.23SS	6.47MS	6.77SS		7.02MS	7.22SS	7.45SS	7.75MS	
	6.27SS	6.60vw	6.73MS	6.92WS	7.11sh	7.24MS	7.50vw	7.60MS	7.90WS
						[7.37MS]			
	6.25MS		6.68SS		7.02vw		7.48WS		8.00MS
	6.35SB	6.62SB			7.03SS		7.45vw	7.60vw	
6.08SS	6.37SS	6.60SS	6.85MS		7.08SS	7.37MS	7.55MS	7.77vw	7.90vw
	6.25SB	6.55SS	6.65MS	6.90MS	7.07SS	7.40MS	7.55MS	7.73WS	8.00vw
			[6.77vw]						
6.11SS	6.27WS	6.57WS	6.67sh	6.84SB	7.06SS	7.43SS	7.60MS	7.84MS	7.98SS
6.15sh	6.33SB		6.63SS	6.90WS	7.09SS	7.40MS	7.58MS	7.85vw	8.04MS
6.20sh	6.33SB		6.65MS	6.92vw	7.10SS	7.40MS	7.57MS	7.90WS	8.07WS
6.16MS	6.33SS		6.74SB	6.88sh	7.10SS	7.24MS	7.47MS	7.72MS	7.89vw
	6.34SS		6.64MS	6.88MS	7.13MS	7.27sh	7.48WS	7.61WS	7.82WS
				6.95sh					
	6.33SS		6.63MS	6.85sh	7.13SS				
6.18sh	6.31SB	6.42sh	6.70SS	6.88vw	7.08MS	7.40MS	7.46sh	7.58MS	7.83MS
				6.95vw	7.17MS				
6.18sh	6.35SB	6.40sh	6.65SB	6.87WS	7.02MS	7.40MS	7.59MS	7.85WS	8.00MS
					7.11MS				

at 6.67 and 6.85 μ. The absence of the N–H deformation frequency in the spectrum of isovaline, and its shift in position and attenuation in *t*-leucine must be a function of either van der Waals repulsive forces or steric conflict, or a combination of both. The spectrum of isovaline in the 3 μ region is further distinguished by the possession of bands at 2.97 and 3.18 μ, that of *t*-leucine in the 6 μ region by bands at 6.45 and 6.53 μ. Peaks in the former region are not encountered in number except in the amino acids of group IV (the ω-hydroxy compounds) and group VI (the ω-amino compounds), and in the latter region except for the compounds of group IV. The spectra of valine and of the isoleucines resemble each other as might be expected; the presence of a sharp and discrete band at 6.84 μ for the latter is the distinguishing difference between the two in the 2.0 to 7.5 μ region.

Compounds in Groups IV, VI and VII.—These amino acids constitute, respectively, the ω-OH, ω-COOH and ω-NH_2 substituted straight chain α-amino acids. The electronegative groups act upon the N–H stretching vibration of the $-NH_3^+$ group with a resulting shift in its absorption to longer wave lengths as they approach the α-amino group. Thus, in serine, homoserine, pentahomoserine and hexahomoserine, this vibration absorbs, respectively, at 4.92, 4.80, 4.73 and 4.7 μ; in aspartic acid at 4.85 and 5.25 μ, and in aminoadipic acid at 5.20 μ; and in α,β-diaminopropionic acid at 5.08 μ, in α,γ-diaminobutyric acid at 4.97 μ, and in lysine at 4.80 μ. As the number of methylene groups between the α-amino group and the electronegative group increases, the degree of interaction and magnitude of the wave length shift decreases.

All of the amino acids of groups IV, VI and VII (with exception of lysine) possess the 3.3 μ band in common. The ω-OH amino acids in addition possess absorption peaks below 3.3 μ, except for allothreonine. The lactone of homoserine, unlike the free amino acid, does not absorb below 3.3 μ, but does possess an extra band at 3.52 μ. Furthermore,

the lactone does not absorb at 3.7 μ nor at 3.9 μ, nor does its N–H motion absorb at 4.7 μ but instead is shifted toward longer wave lengths in the region of 5.1 μ. This lactone is an internal ester, and the loss of the <3 μ OH-vibration frequency is not surprising. A shift in the N–H 4.7 μ region to that of 5 μ is also apparent in the spectra of the phenylserines, an effect not entirely due to interference by the β-ring substituent inasmuch as all of the compounds in group III absorb normally in the 4.7 μ region. In group VII, arginine and citrulline have bands 6.3 and 6.6 μ. The former group but not the latter absorb at 4.0 and 6.9 μ.

Although the 4.7 μ N–H stretching frequency band occurs as such in most of the compounds of group IV and group VII, or else is recognizably shifted to longer wave lengths, most of the compounds in group VI apparently are lacking in this peak. Thus, glutamic acid has no band in this region, nor has aminotricarballylic acid or the corresponding pyrrolidones and lactones. The aminotricarballylic acids and their pyrrolidones and lac-

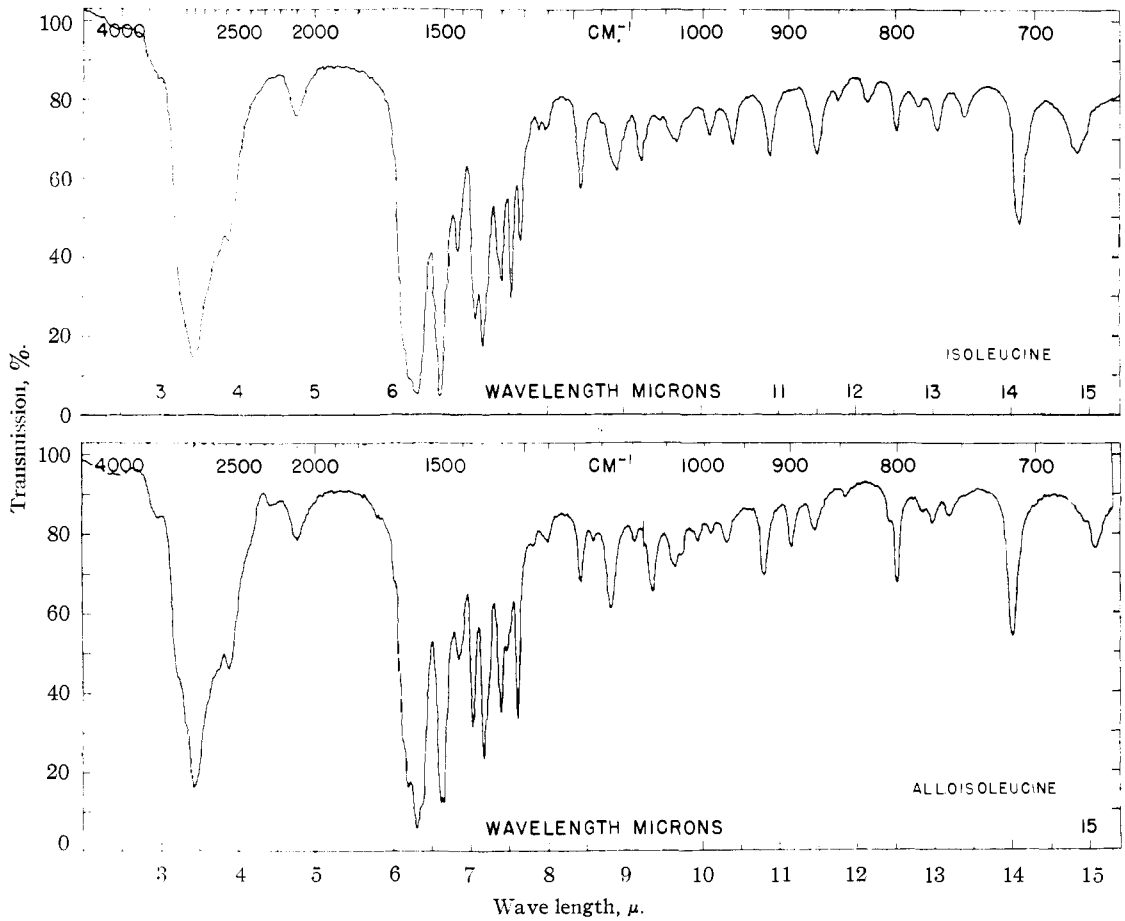

Fig. 1.—Infrared absorption spectra of isoleucine and alloisoleucine.

below 3.3 μ. The strong sharp absorption band at 3.0 μ in these compounds is tentatively assigned to the N–H stretching frequency of the NH_2 group in the ureido and guanido radicals.

An interesting group of compounds in which absorption peaks occur at less than 3.3 μ consists of aminotricarballylic acid and of the pyrrolidone forms of this compound and of its allostereomer, as well as of the corresponding lactones of the isocitric acids derived therefrom (group VI). The pyrrolidones and lactones do not absorb at 3.3 μ nor at 4.7, tones as well as aspartic acid, and the unsubstituted succinic and tricarballylic acids all have instead sharp bands at 5.9 μ which are probably related to the vibrational frequencies of an un-ionized carboxyl group. The 5.9 μ band, related to the γ(C=N) or γ(C=O) frequency, occurs also in the spectra of arginine and of citrulline. Ornithine possesses no band in the 4.7 μ region. The sharp band at 5.73 μ may represent un-ionized –COOH, in view of the fact that this compound was studied as the dihydrochloride, unlike the other diamino

acids which were studied as the monohydrochlorides.

The polycarboxylic acids may be expected to contain not only ionized but also un-ionized carboxyl groups. The latter absorb at 5.76, 5.80, 5.92, 6.00 and 6.07 μ. One or more of these bands is to be found in the spectra of the compounds in group VI. All of these compounds when examined in D_2O exhibit three strong, sharp absorption peaks at 5.83 ± 0.03 μ, at 6.19 ± 0.04 μ, and at 7.00 ± 1.0 μ. The 5.83 μ band is due to the γ(C=O) of the un-ionized carboxyl group, and the other two bands, respectively, to the antisymmetrical and symmetrical γ(C=O) of the carboxylate ion.

Ornithine and lysine possess a 6.6 μ band related to the δ(N–H) frequency. With α,β-diaminopropionic acid, wherein the two amino groups are closest in structure, this band is seemingly replaced by two bands at 6.47 and 6.77 μ, and if this is so the splitting of the band in this region may well result from the inductive interaction of the two adjacent amino groups. In α,γ-diaminobutyric acid the influence on the δ(N–H) motion is not so marked; two absorption bands are present at 6.60 and 6.73 μ, but the peak at 6.60 μ is very weak. The spectrum of histidine also suggests a similar inductive effect, for there is a strong broad absorption band at 6.84 μ with a weak shoulder at 6.67 μ. This band and shoulder are tentatively assigned to a N–H deformation motion.

In the region above 6 μ, hexahomoserine exhibits a spectrum which for all practical purposes is nearly identical with that of the prototypical amino acids in group I. The symmetrical and antisymmetrical stretching frequencies of the ionized carboxyl absorb at 7.14 and 6.36 μ, respectively, the N–H deformation motion at 6.65 μ, and C–H bending at 6.96 μ. As the ω-OH group approaches the α-carbon configuration, as in pentahomoserine, homoserine and serine, there is a progressive shift toward the shorter wave length in the 6.96, 7.14, 7.42 and 7.59 μ bands of hexahomoserine. Even more dramatically, the N–H deformation motion at 6.65 μ in hexahomoserine is shifted to 6.57 μ in pentaho-

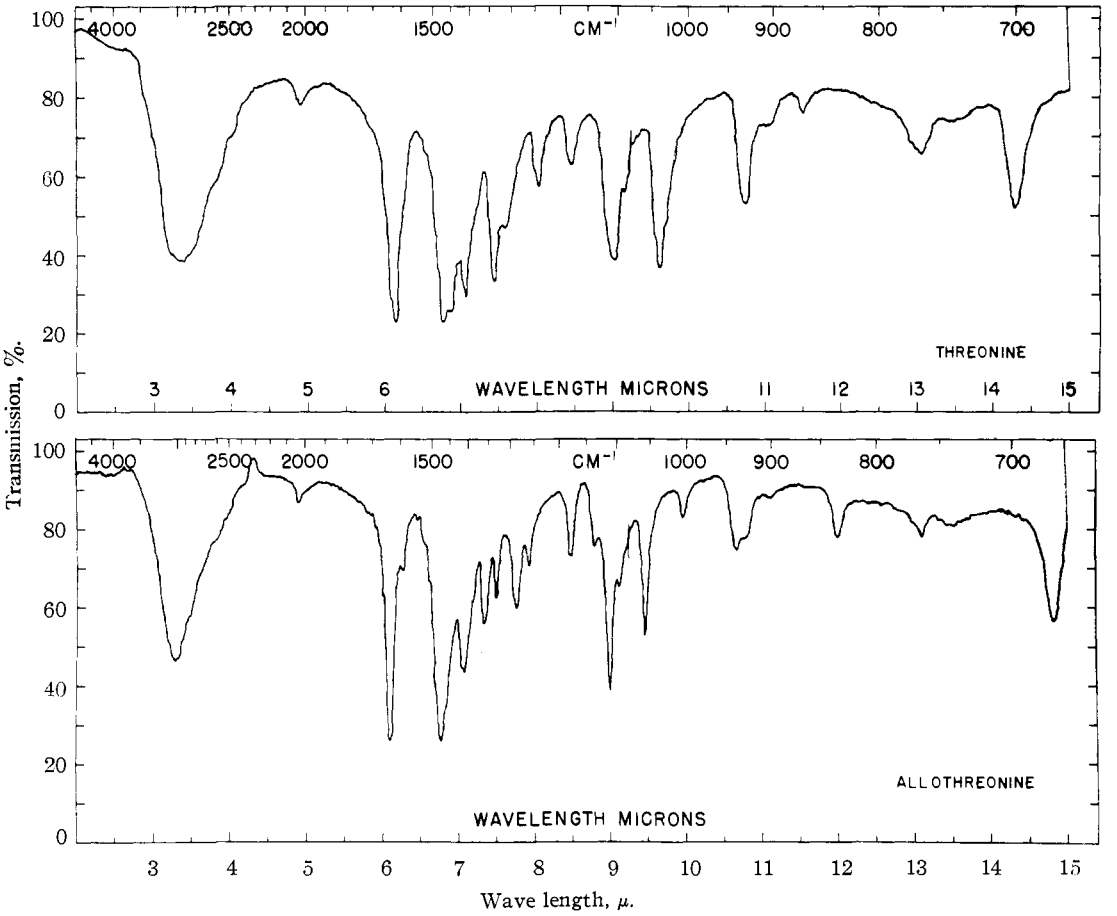

Fig. 2.—Infrared absorption spectra of threonine and allothreonine.

moserine, then to 6.50 μ in homoserine, and seemingly disappears altogether in serine. Thus, again, as the functional groups in a homologous series of amino acids approach each other spatially, some type of interaction occurs resulting in a progressively increasing perturbation of the N–H deformation motion.

Compounds in Group III.—The compounds in this group are characterized by having phenyl or cyclohexyl groups on the α-carbon atom of glycine (aminophenylacetic acid or aminocyclohexylacetic acid) or on the β-carbon atom of alanine (phenylalanine or aminocyclohexylpropionic acid) and *p*-hydroxyphenyl or indole rings on the β-carbon atom of alanine (tyrosine or tryptophan). Comparison of corresponding phenyl and cyclohexyl amino acids reveals that aminophenylacetic acid differs from aminocyclohexylacetic acid in lacking the 3.3 and 7.5 μ bands, and in possessing a 6.45 μ band; all other bands are shared in common. On the other hand, the differences between phenylalanine and aminocyclohexylpropionic acid are due to the lack of the 3.9, the 6.9 and 7.4 μ bands, and the possession of a 6.17 μ band. There seems to be no unique effect produced on the spectrum by converting a phenyl to a cyclohexyl group. Tryptophan differs considerably in its spectrum from that of the other members of this group, in possessing a 5.98 μ band which appears in the spectra only of the compounds in group VI and VII, and in lacking the 6.2 and 6.6 μ bands. Phenylalanine, tyrosine and aminophenylacetic acid absorb strongly at 6.2 μ, a region to which vibrations of the phenyl radical have been assigned.[26–28] It must be pointed out however, that aminocyclohexylacetic acid also absorbs in this region, and that tryptophan does not.

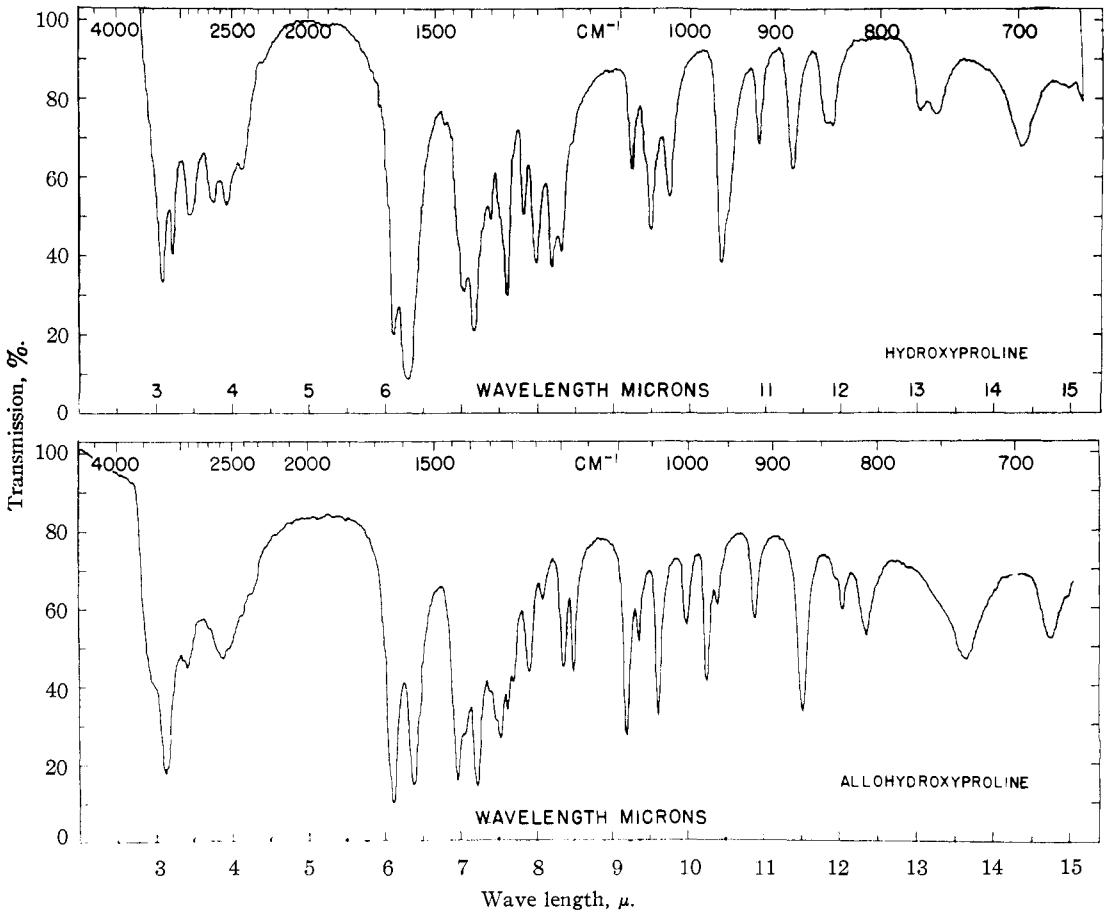

Fig. 3.—Infrared absorption spectra of hydroxyproline and allohydroxyproline.

Compounds in Group V.—These compounds all have a basic imino nitrogen bond in a five-membered hydrocarbon ring. It is of interest

(26) K. Lunde and L. Zechmeister, *Acta Chem. Scand.*, **8**, 1421 (1954).

(27) R. S. Rasmussen and R. R. Brattain, *J. Chem. Phys.*, **15**, 131 (1947); R. S. Rasmussen and P. S. Zucco, *ibid.*, **15**, 135 (1947).

(28) N. Herzfeld, C. K. Ingold and H. G. Poole, *J. Chem. Soc.*, 316 (1946).

therefore to scrutinize particularly those regions of the spectrum assigned to N–H motions, and it is significant therefore to note that the 4.7 μ region is completely absent for all three compounds in this group, and the 6.6 μ band is present only in the spectrum of proline. The 7.1, 7.3 and 7.5 μ bands are shared by all three. The 3.1 μ band, present in the hydroxyprolines but not in the spectrum of proline, probably reflects the presence of the γ-OH group in the former.

tra of the compounds in this group.[29] All of the compounds, like most of those in groups I and II, possess a weak absorption band at about 3.9 μ.

Diastereoisomeric Pairs of α-Amino Acids.—No difference in the absorption of the L- and D-forms of these amino acids between 2 and 15 μ is apparent, and this was found to be true of the solid state spectra over this wave length for each of the 53 optically enantiomorphic pairs of amino acids

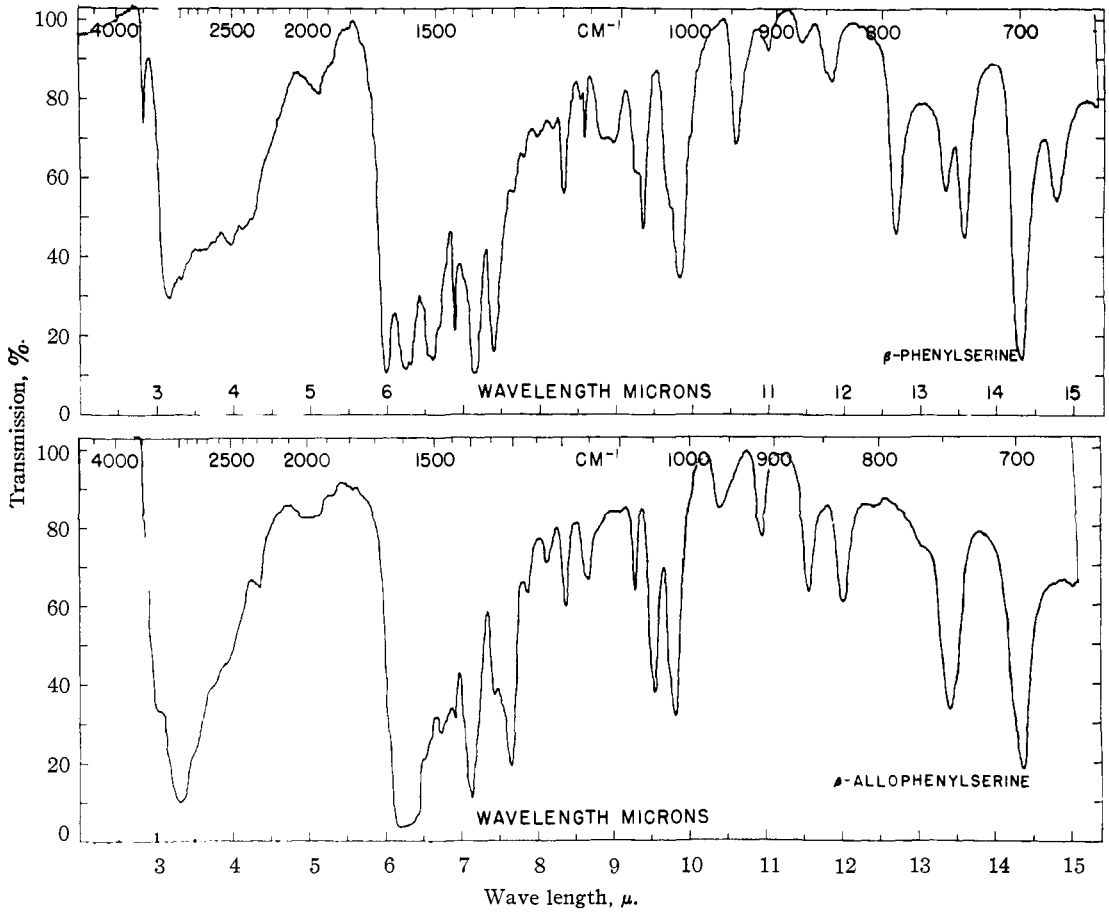

Fig. 4.—Infrared absorption spectra of β-phenylserine and β-allophenylserine.

Compounds in Group VIII.—The spectra of cystine and cysteine are indistinguishable from the 3 μ to the 6 μ regions. At higher wave lengths the spectra diverge, for the latter compound does not possess the 6.1, 7.3 and 7.5 μ bands characteristic of the former. From 3.9 μ to higher wave lengths, *i.e.*, 7.5 μ, the spectra of methionine and ethionine, of cystine and homocystine, and of S-benzylcysteine and S-benzylhomocysteine, are nearly identical. The presence of the sulfur atom does not appear to induce any striking effect on the spectra examined. This complete correspondence in the solid state spectra of the optical antipodes permits the spectra of the diastereoisomeric pairs to be interpreted solely on the basis of the difference in configuration of the members of each pair about the asymmetric β-carbon atom. The spectral curves for this category of amino acids, together with the pyrrolidone and lactone forms derived from aminotricarballylic acid are given in Figs. 1–7.

(29) *Cf.* N. Fuson, M.-L. Josien and R. L. Powell, THIS JOURNAL, **74**, 1 (1952).

Isoleucine–Alloisoleucine.—Of all the diastereomeric pairs studied, the spectra of isoleucine and alloisoleucine most closely resemble each other. From 2 to 8 μ, the spectra of these two compounds are practically identical, and this striking similarity in the region of the fundamental stretching frequencies of their solid state spectra can only be interpreted as indicating that the difference in spatial configuration about the respective β-center of asymmetry must produce little if any change in the vibrating electrical charge coupled with each nuclear vibration. It is of interest in this connection that the optical rotatory power of isoleucine and alloisoleucine is very nearly the same, a condition which holds for no other pair of diasteromeric amino acids studied.[11] The first marked difference in the spectra of isoleucine and alloisoleucine appears in the 8 μ region, and from here to 15 μ the spectra of these compounds are sufficiently different to permit their use for analytical purposes. The optical configuration of the β-center in L-isoleucine is L, that of L-alloisoleucine would naturally be D.[30-32]

Threonine–Allothreonine.—The optical configuration of the β-center in L-threonine is D, that of L-allothreonine is L.[33] The infrared spectra of these two isomers reveal several differences. Thus, threonine lacks the 6.3 μ band and allothreonine lacks the 6.9 μ band. On the other hand, threonine possesses a strong band at 3.2 μ which is not observed in the spectrum of allothreonine. Indeed, allothreonine is the only hydroxyamino acid studied in whose spectrum this band is not observed. Both compounds however absorb in the 3.3 μ region. Beyond 8 μ, there are a larger number of discrete bands in the spectrum of allothreonine than of threonine.

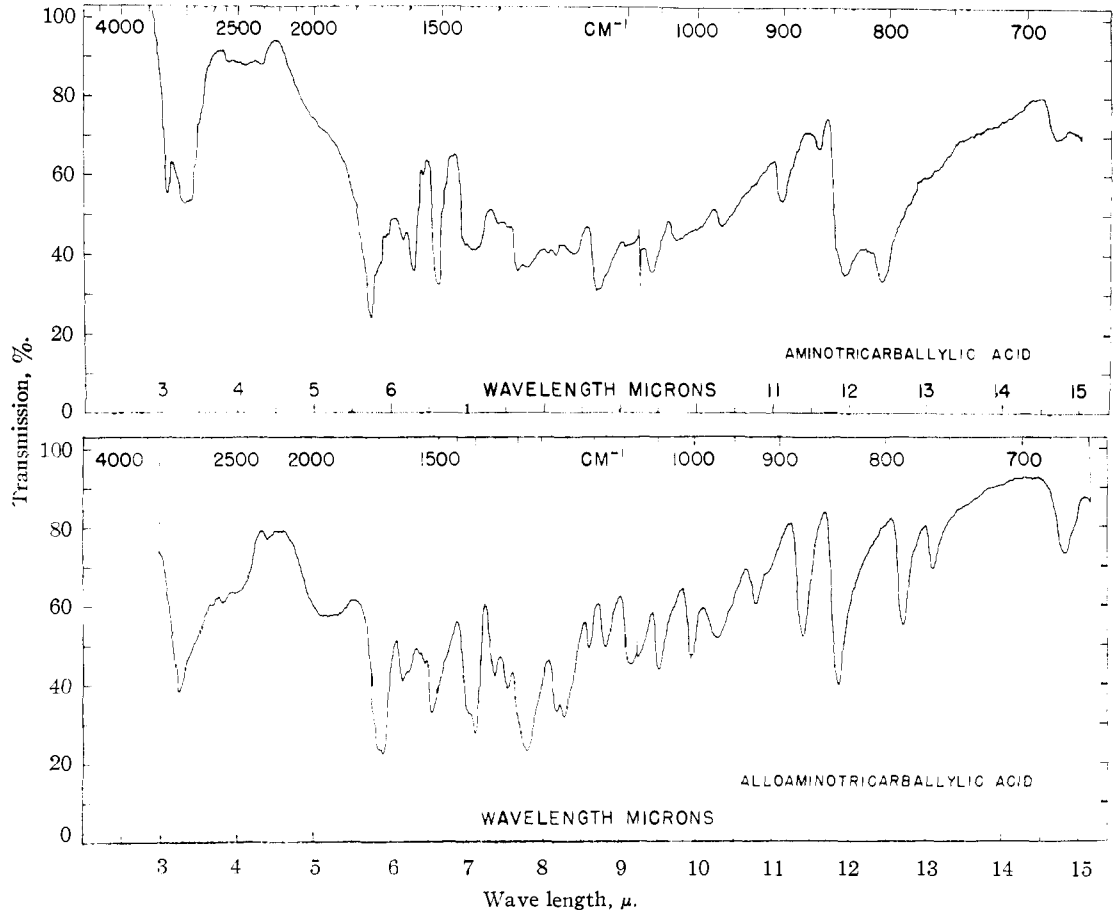

Fig. 5.—Infrared absorption spectra of aminotricarballylic and alloaminotricarballylic acid.

Hydroxyproline–Allohydroxyproline.—In the L-form of hydroxyproline the hydroxyl group of the γ-asymmetric center is *trans* to the α-carboxyl group.[34,35] In the 3.0 μ region, the spectra of this pair show differences of some magnitude. The allo isomer absorbs at 3.17 μ, assigned to O–H and N–H stretching motions, and the wave length at which this band absorbs suggests considerable perturbation of these vibrations. In the spectrum of hydroxyproline there are two strong sharp bands in

(30) S. Ståhlberg-Stenhagen and E. Stenhagen, *Arkiv. Kemi Mineral. Geol.*, **24B**, 1 (1947).

(31) J. Trommel, *Proc. Acad. Sci. Amst.*, Series B, **56**, 272 (1953); *ibid.*, Series B, **57**, 364 (1954).

(32) M. Winitz, S. M. Birnbaum and J. P. Greenstein, THIS JOURNAL, **77**, 3106 (1955).

(33) C. E. Meyer and W. C. Rose, *J. Biol. Chem.*, **115**, 721 (1936).

(34) A. Neuberger, *J. Chem. Soc.*, 429 (1945).

(35) J. Zussman, *Acta Cryst. Camb.*, **4**, 72 (1951).

this region at 3.10 and 3.23 μ also assigned to N–H and O–H stretching motions. The 3.7 and 6.3 μ bands found in the spectrum of hydroxyproline are not observed at these positions in that of allohydroxyproline. These bands refer, respectively, to N–H and C–H and to antisymmetrical $-COO^-$ vibrations. The O–H deformation motion in hydroxyproline absorbs at 9.47 μ; the corresponding vibration in the allo isomer absorbs some 0.13 μ toward the shorter wave length. The 6.9 μ band (C–H bending motion) is not seen in the spectrum of hydroxyproline, but there are two bands in the 7.1 μ spectral region of this compound. Beyond 8 μ, the spectra of the two diastereomers differ very considerably.

Phenylserine–Allophenylserine.—Here again, the β-carbon in L-phenylserine has the D-configuration, that of L-allophenylserine the L-configuration.[36] Phenylserine has three bands in the 3 μ spectral region, allophenylserine has two. The former compound also has two bands in the 6.3 and 6.6 μ region, instead of only one in each region for the latter compound. Allophenylserine is lacking the 3.95 μ band which is present in phenylserine. There seems little doubt that in the 2 to 7.5 μ region, phenylserine possesses more bands than does its allo diastereomer, and this difference also holds for the spectra beyond 8 μ as well.

Aminotricarballylic Acid–Alloaminotricarballylic Acid.—The optical configurations at the β-carbon atoms of these compounds is not known at the present time. These α-amino acids possess three carboxyl groups on adjacent carbon atoms, and may be considered from one point of view as β-carboxylated glutamic acids. There are marked differences in the spectra of aminotricarballylic and alloaminotricarballylic acids, the latter showing many more discrete bands than the former over the entire spectral range studied. The allo isomer possesses two resolved absorption bands at 5.8 μ, its diastereomer only one band, all of which are assigned to the motions of the un-ionized carboxyl group or groups. It may be suggested therefrom that the spatial positions of the carboxyl groups in aminotricarballylic acid relative to each other are

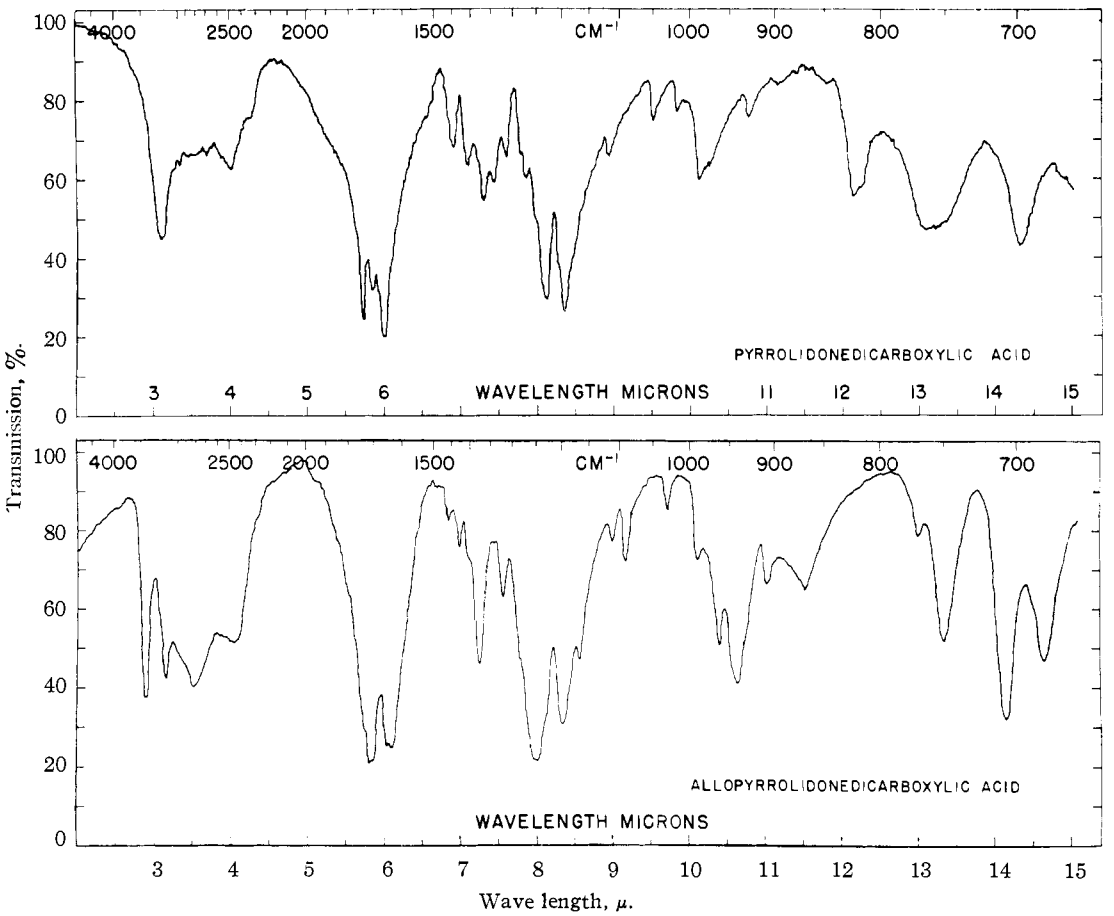

Fig. 6.—Infrared absorption spectra of pyrrolidinedicarboxylic acid and allopyrrolidinedicarboxylic acid.

(36) W. S. Fones, *Archiv. Biochem. Biophys.*, **36**, 486 (1952).

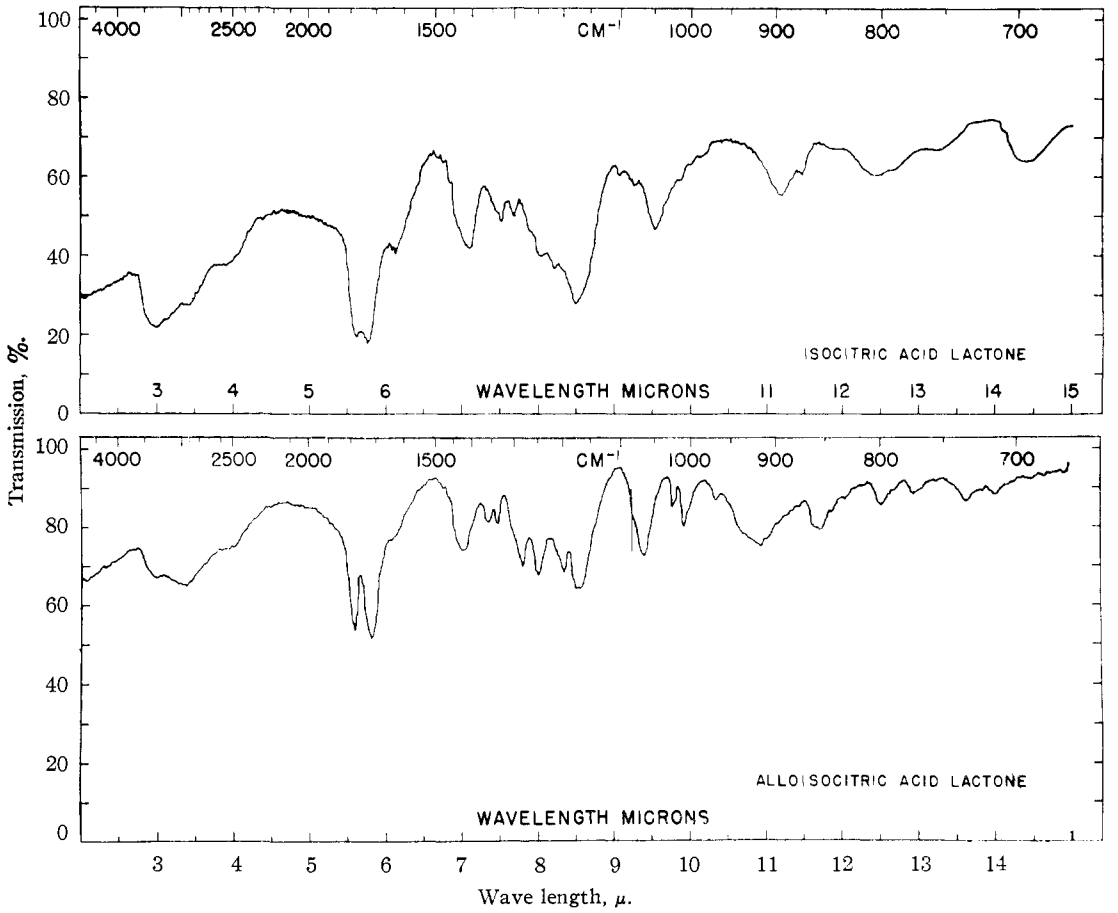

Fig. 7.—Infrared absorption spectra of isocitric acid lactone and alloisocitric acid lactone.

so oriented that perturbation of their carbonyl stretching frequencies does not occur. The antisymmetrical γ(C=O) vibration at 6.3 μ occurs in the spectrum of aminotricarballylic acid, and not in that of its allo isomer unless it is assumed that it is shifted toward the lower wave length at 6.17 μ due to perturbation of this vibration. Neither diastereomer possesses the 4.7 μ band characteristic of N–H vibration, although it is possible that in the spectrum of the allo form this band has been shifted to 5.25 μ. Both diastereomers readily and quantitatively form the corresponding pyrrolidonedicarboxylic acids in aqueous solution, but the allo form accomplishes this much more rapidly.

Pyrrolidonedicarboxylic Acid Derivatives of Aminotricarballylic Acid–Alloaminotricarballylic Acid.—In these compounds, the α-amino nitrogen of the amino acid becomes an amide nitrogen due to ring closure with the γ-carboxyl group. The spectrum of the derivative of aminotricarballylic acid has two bands in the un-ionized –COOH region at 5.8 μ, that of the allo form but one. This is the converse of the free amino acids. Neither compound shows the presence of bands characteristic of N–H motion in NH_3^+, and neither shows a band at 6.3 μ.

Lactone Derivatives of Isocitric Acid–Alloisocitric Acid.—The optically active isocitric acids were prepared from the isomers of aminotricarballylic acid without change in configuration, and were isolated as the lactones.[37] They differ from the corresponding pyrrolidonedicarboxylic acids in possessing an oxygen in the ring instead of nitrogen, and hence are internal esters rather than internal amides. The spectra of the pair of diastereomeric lactones show a surprising degree of similarity in the 2 to 7 μ region. Only at 7.36 μ, a band possessed by the lactone of alloisocitric acid but not by its diastereomer, is there a visible difference in this spectral range between the two. Beyond 8 μ the spectra are quite dissimilar, that of the isocitric acid lactone being rather ill-defined.

The sharp band in the 14 μ region, arising in part from a C–H rocking vibration,[38] and later suggested by Blout and Linsley as due also in part to the N–H grouping,[39] occurs in all of the amino acids studied. There is no evidence of this peak in the spectrum of alloisocitric acid lactone, while in that of its diastereomer it occurs as a broad band at about 14.3 μ.

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(37) J. P. Greenstein, N. Izumiya, M. Winitz and S. M. Birnbaum, THIS JOURNAL, **77**, 707 (1955).

(38) N. Sheppard and G. B. B. M. Sutherland, *Nature*, **159**, 739 (1947).

(39) E. R. Blout and S. G. Linsley, THIS JOURNAL, **74**, 1946 (1952).