

Investigations of the Infra-Red Absorption Spectra of Some Amino-Acids between 2 and 15 μ

LENNART LARSSON

Institute for Cell Research, Karolinska Institutet, Stockholm, Sweden

This investigation has been made to evaluate the possibility of determining directly the various amino-acids composing the proteins without preliminary chemical disintegration, as, for example, hydrolysis. This direct approach is important in the study of endocellular protein metabolism. The purpose of this study was to determine, whether the infrared absorption spectra of the amino-acids contained absorption bands specific for the different amino-acids, likewise whether these bands could be found again in the spectrum of a protein or peptide. From earlier investigations of the infra-red absorption spectra of amino-acids between 0.5 and 8.0 μ made by Heintz^{1, 2} and Vlès³, it appears that the difference between the spectra of the amino-acids in this range is not sufficient to allow their separate identifications. One might anticipate that the absorption bands specific for a substance are, however, to be found in a wavelength range above 8 μ . Heintz and Vlès examined the spectra of the amino-acids, dissolved in water or deposited as a thin powder layer on a rock salt base, the latter technique used also by Lenormant^{4, 5, 6}. Because the infrared absorption of water is very great and coincides with that of the amino-acids, the former method cannot be considered satisfactory. The powder technique is also unsatisfactory in certain respects, which will later be treated. Many infra-red spectra of amino-acids have been recorded by Wright⁷ in the range, 3 to 24 μ , for powder layers on potassium bromide plates using a potassium bromide prism. Such a prism, however, has relatively poor resolution particularly in the range below 10 μ .

In an attempt to meet above difficulties the infra-red absorption spectra between 2 and 15 μ , of five amino-acids, closely related compounds and one dipeptide, have been studied. Similar investigations have recently been made by Darmon and Sutherland^{8, 9}.

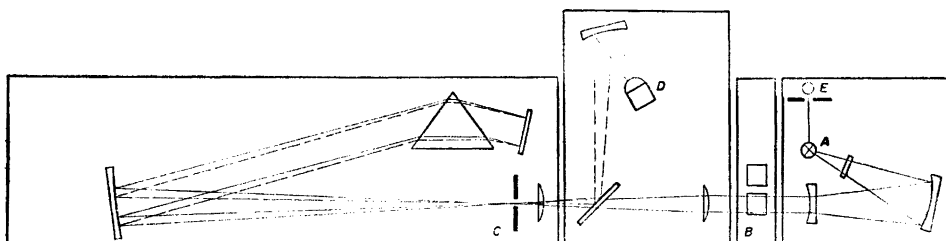


Fig. 1. Schematic optical path of the spectrophotometer. A. Radiation source. B. Liquid cell compartment. C. Slit. D. Photo-receiver, Bolometer. E. Glower regulating phototube.

APPARATUS

For these examinations a Beckman Infra-red Spectrophotometer was used to record spectra continuously and automatically. It was equipped with rock salt optics and rock salt prism. The latter is most suitable to this object, because it provides the best combination of dispersion and transmission range. A schematic drawing of the optical path of the spectrophotometer is shown in Fig. 1. The radiation source is an efficient Nernst glower, the radiation output of which is automatically held constant within 0.1 % by a special photo-electronic regulator.

The entire instrument is maintained constantly at the temperature of $25.0 \pm 0.1^\circ \text{C}$ by means of integral coils and chambers through which oil from a constant temperature bath is circulated. With such control localized heating cannot occur, nor are temperature corrections necessary.

The reproducibility of the wavelength is about 0.005μ , but at wavelengths shorter than 6μ , the reproducibility decreases proportionately to the reduction in dispersion of its rock salt prism. The wavelength scale of the instrument was calibrated to the CO_2 band at 4.224μ , after which check was made to establish that the scale coincided with the CO_2 band at 13.87μ .

Because the radiation intensity successively decreases at longer wavelengths, it is necessary to compensate this decrease by increasing the slit width at given intervals. This was done according to the scheme below:

Wavelength interval in μ	Slit width in mm	Wavelength interval in μ	Slit width in mm
1.0—3.2	0.10	8.7—10.5	0.50
3.2—5.9	0.15	10.5—13.0	0.60
5.9—6.7	0.20	13.0—15.0	0.70
6.7—8.7	0.40		

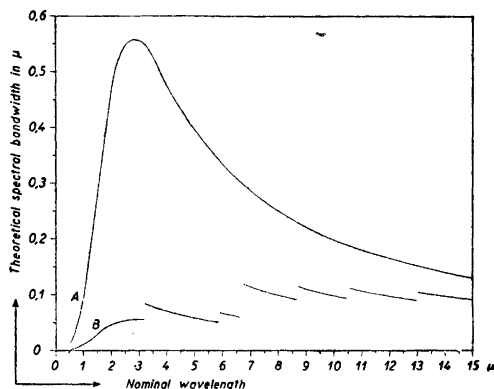


Fig. 2. Resolving power for Beckman spectrophotometer with rock salt prism. A. Theoretical curve for 1 mm slit width. B. From curve A calculated curve for the slit widths used in the examinations.

The resolving power, which is calculated from a theoretical curve for a Beckman spectrophotometer for 1 mm slit width, is illustrated in Fig. 2, which shows that the band width is about 0.1 μ in these examinations.

EXPERIMENTS AND RESULTS

For recording the infra-red spectra, it proved best to use suspensions of the amino-acids, because there are no suitable solvents for them. The powder technique, which was applied by Heintz^{1,2}, Vlès³ and many others, has the disadvantage that the absorption curves vary markedly with the particle size¹⁰. If particle size is approximately the same as the wavelength of the radiation used for study, the radiation will be scattered rather than transmitted, and a false absorption maximum will appear.

The suspensions were prepared by grinding the substances in a powerful, mechanical glass mortar with paraffin oil, a very good suspension medium owing to its high viscosity and relatively weak absorption in infra-red. The particle size in the suspensions must be as small as possible, for in this instance also the particle size will, to a certain degree, influence the transmission. With the suspension method mentioned above the average particle size was about 1.2 μ , determined by microphotographic measurement. An attempt was made to suspend the amino-acids in paraffin oil with supersound at frequencies between 0.4 and 0.7 Mc/sec^{11,12}. This failed, probably because the high viscosity of the paraffin oil impeded the cavitation effect. The amino-acid suspension, the concentration of which was about 5 %, was introduced with a record syringe into a rock salt cell. The light path length of the cell was 0.40 mm and the quantity of the suspension used to fill the cell was approxim-

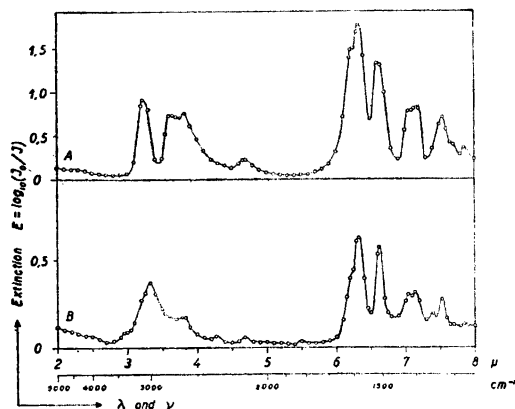


Fig. 3. Infra-red absorption spectra of L-valine. A. L-Valine suspended in paraffin oil. B. Sublimated L-valine.

ately 0.5 cc. The substances used were obtained from F. Hoffmann-La Roche & Co.

Paraffin oil absorbs strongly in the characteristic regions of the C-H frequencies (3.45μ , 6.85μ and 7.25μ), but the remainder of the spectral range will be available. To ascertain the errors in the absorption curves, caused by the absorption of the paraffin oil itself, the absorption curve of suspended valine was compared with that of valine sublimated on a rock salt plate (layer thickness about 4μ). Fig. 3 shows that the two curves differ only in the region between 3.35 and 3.55μ . On the other hand no differences are detectable at 6.85 and 7.25μ , which is very surprising. In spite of the small particle size, obtained by the sublimation of amino-acids, one cannot escape the disturbing effect of the scattering of the radiation. Above 8μ the absorption of the paraffin oil is very weak.

Comparisons of the spectra of various compounds containing the same radical have shown that absorption bands occurring at certain frequencies can be correlated with specific atomic configurations within the molecule, as, for example; C—H, C=O, O—H, N—H, *etc.*^{13, 14}. For simple or highly symmetrical molecules the frequencies of the absorption bands can be calculated mathematically, but when one considers structurally complicated compounds such as amino-acids, it is necessary to resort to the empirical method. By successive elimination of the radicals in a molecule it is often possible to discover the specific absorption bands and from these the corresponding frequencies or wavelengths. Actually only a few of the observed bands can be correlated in this manner.

Fig. 4 shows the infra-red absorption bands of five amino-acids, one amine and its hydrochloride, one carboxylic acid and its sodium salt. From these

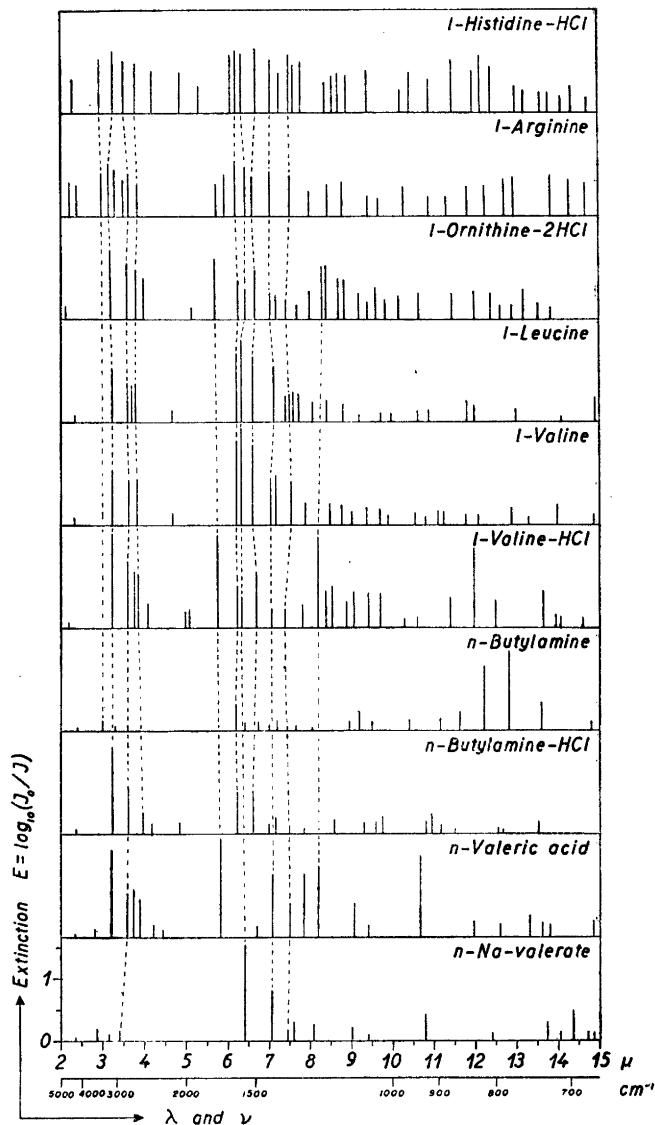


Fig. 4. Infra-red absorption bands of the five recorded amino-acids, one amine and its hydrochloride, one carboxylic acid and its sodium salt. The dotted lines between the bands indicate that these are common to the amino-acids and characteristic of a certain atomic configuration.

spectra one can observe the absorption frequencies of some common radicals and likewise the range within which the specific bands of the various amino-acids are to be found.

From the spectra, shown in Fig. 4, it is evident that the absorption bands characteristic of amino-acids are concentrated within two ranges: one between

2.9 and 4.0 μ , and the other between 5.7 and 7.6 μ . Within the first-mentioned range the absorption frequencies of the bondstretching vibrations of the

radicals $\begin{array}{c} | \\ -\text{C}-\text{H} \\ | \end{array}$, $\begin{array}{c} > \\ \text{C} \\ < \end{array} \begin{array}{l} \text{H} \\ \text{H} \end{array}$, $\begin{array}{c} \text{H} \\ | \\ -\text{C}-\text{H} \\ | \\ \text{H} \end{array}$, $\begin{array}{c} \text{H} \\ | \\ -\text{N} \\ | \\ \text{H} \end{array}$, $\begin{array}{c} \text{H} \\ | \\ -\text{N}^+ \\ | \\ \text{H} \end{array}$ and $-\text{O}-\text{H}$ will occur.

Within the other range there are absorption frequencies of the bondstretching vibrations of $\begin{array}{c} > \\ \text{C} \\ < \end{array} \text{O}$ and $\begin{array}{c} \text{O} \\ | \\ -\text{C} \\ | \\ \text{O} \end{array}$ and of the bondbending vibrations of the former radicals¹⁵.

Absorption in the region 2.90—3.00 μ (3450—3330 cm^{-1}). Butylamine shows at 3.00 μ an absorption band, which recurs in the spectra of histidine hydrochloride at 2.94 μ and of arginine at 2.98 μ . This seems to be caused by the bondstretching vibrations in $\begin{array}{c} > \\ \text{N} \\ < \end{array} \text{H}$.

Absorption in the region 3.15—3.30 μ (3180—3030 cm^{-1}). In this region butylamine hydrochloride has a strong absorption band identical to that in the following amino-acids: histidine hydrochloride (3.27 μ), arginine (3.17 μ), ornithine dihydrochloride (3.20 μ), leucine (3.23 μ), valine (3.23 μ) and valine hydrochloride (3.23 μ). This absorption arises from the bondstretching vibrations in the charged radical $\begin{array}{c} + \\ \text{NH}_3 \end{array}$.

In the same region valeric acid also has a strong absorption band caused by the bondstretching vibrations in $-\text{O}-\text{H}$. This band, which is expected to occur likewise in the spectra of amino-acid hydrochlorides, cannot, however, be distinguished from the absorption of $\begin{array}{c} + \\ \text{NH}_3 \end{array}$.

Absorption in the region 3.40—3.65 μ (2940—2740 cm^{-1}). In all the examined compounds a broad absorption band is present in this region. This can be assigned to the symmetrical and antisymmetrical bondstretching vibrations in $\begin{array}{c} > \\ \text{CH}_2 \end{array}$ and $-\text{CH}_3$. The different bands cannot be discriminated, partly because they lie so close to one another and partly because of the disturbing absorption of the paraffin oil.

Absorption in the region 3.80—4.00 μ (2630—2500 cm^{-1}). This absorption seems to be due to the bondstretching vibrations in the charged radical $\begin{array}{c} + \\ \text{NH}_3 \end{array}$, which appears from the absorption band of butylamine hydrochloride at 3.96 μ . For the amino-acids this band occurs at somewhat shorter wavelengths: histidine hydrochloride (3.81 μ), arginine (3.86 μ), ornithine dihydrochloride (3.81 μ), leucine (3.81 μ), valine (3.83 μ) and valine hydrochloride (3.86 μ).

Absorption in the region 5.70—5.85 μ (1750—1710 cm^{-1}). It has been shown that the carboxylic acids have an absorption band at approximately 5.8 μ , this arising from the bondstretching vibrations in the uncharged group $-\text{COOH}$. This band recurs in the spectra of the hydrochlorides of the amino-acids, because the amino group here is blocked, and no zwitterion can be

formed. For ornithine dihydrochloride this band lies at 5.72μ and for valine hydrochloride at 5.77μ . Histidine hydrochloride, however, lacks this band, because the proton here is bound to the nitrogen of the imidazole nucleus, and a charged carboxylic group is formed.

It should be noted that arginine also has an absorption band at 5.75μ , but this cannot be caused by the carboxylic group.

Absorption in the region $6.15-6.30 \mu$ ($1615-1590 \text{ cm}^{-1}$). This band will be assigned to the bondbending vibrations in the charged radical $^{\pm}\text{NH}_3$. This absorption recurs in the spectra of butylamine hydrochloride (6.24μ), histidine hydrochloride (6.22μ), arginine (6.20μ), ornithine dihydrochloride (6.27μ), leucine (6.22μ), valine (6.22μ) and valine hydrochloride (6.23μ).

Butylamine also gives rise to an absorption band at 6.20μ , which may be interpreted as the bondbending vibrations in >N-H . Since histidine hydrochloride and arginine contain >N-H as well as $^{\pm}\text{NH}_3$ radicals, their spectra should show stronger absorption in this range, than that of the other amino-acids. Such is in fact the case.

Absorption in the region $6.30-6.45 \mu$ ($1590-1550 \text{ cm}^{-1}$). Sodium valerate shows here an absorption band at 6.42μ as a result of the bondstretching vibrations in the charged group $-\text{COO}$. This band occurs in the amino-acids: histidine hydrochloride (6.35μ), arginine (6.45μ), leucine (6.34μ), valine (6.33μ), and, peculiarly enough, in ornithine dihydrochloride (6.44μ) and valine hydrochloride (6.35μ). In the latter two the carboxylic group is uncharged and for that reason should cause no absorption in this range. The absorption in these two last-mentioned compounds, however, is much weaker than in the others and may possibly arise from another source.

Absorption in the region $6.60-6.70 \mu$ ($1510-1490 \text{ cm}^{-1}$). Histidine hydrochloride (6.70μ), arginine (6.62μ), ornithine dihydrochloride (6.68μ), leucine (6.62μ), valine (6.62μ) and valine hydrochloride (6.70μ) as also butylamine hydrochloride (6.62μ) have in this region a rather strong absorption band, which seems to depend on the bondbending vibrations in $^{\pm}\text{NH}_3$.

Absorption in the region $7.00-7.15 \mu$ ($1430-1400 \text{ cm}^{-1}$). There is in this region an absorption band common to all the amino-acids studied: histidine hydrochloride (7.06μ), arginine (7.04μ), ornithine dihydrochloride (7.03μ), leucine (7.13μ), valine (7.05μ) and valine hydrochloride (7.06μ) as likewise in the spectrum of valeric acid (7.08μ) and sodium valerate (7.06μ). This absorption is probably assignable to the bondstretching vibrations in the charged as well as uncharged carboxylic group.

Absorption in the region $7.40-7.55 \mu$ ($1350-1325 \text{ cm}^{-1}$). All the compounds examined show here an absorption band, which is to be referred to the bondbending vibrations in C-H .

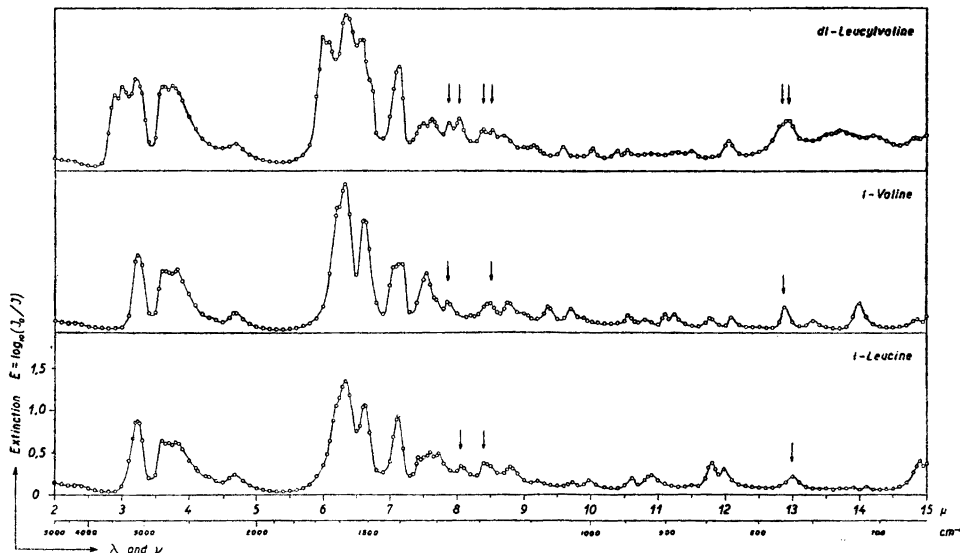


Fig. 5. Infra-red absorption spectra of DL-leucylvaline, L-valine and L-leucine. The arrows indicate the absorption bands specific for the two amino-acids.

Absorption in the region $8.20\text{--}8.30\ \mu$ ($1220\text{--}1200\ \text{cm}^{-1}$). Valeric acid shows at $8.20\ \mu$ a strong absorption band, which is found in valine hydrochloride at $8.21\ \mu$ and in ornithine dihydrochloride at $8.31\ \mu$. (This band is displaced slightly to longer wavelengths because of fusion with a strong band at $8.40\ \mu$). The absorption may possibly be caused by the bondbending vibrations in the radical —O—H .

It is known that the hydrocarbon compounds will absorb strongly at approximately $6.85\ \mu$ ($1460\ \text{cm}^{-1}$) and $7.25\ \mu$ ($1380\ \text{cm}^{-1}$), and that these absorptions are due mainly to various bondbending vibrations in the $\text{—}\overset{\text{H}}{\underset{\text{H}}{\text{C}}}\text{—H}$.

$\text{>C}\begin{matrix} \text{H} \\ \diagdown \\ \text{H} \end{matrix}$, and $\text{—}\overset{\text{H}}{\underset{\text{H}}{\text{C}}}\text{—H}$ groups. These absorption bands, which are likely to occur in the spectra of the amino-acids, have not been distinguishable in the examinations performed, because of the very strong absorption of the paraffin oil exactly in these ranges.

As mentioned before, only a few of the observed bands can be correlated with specific atomic configurations. The principal band components of the spectrum above $7.5\ \mu$ arise from normal modes of skeletal vibrations involving

the C—C and C—N bondstretching vibrations. These bands, which will usually differ among the various amino-acids, can successfully be used for the identification of each compound, as likewise the weak overtone bands and combination bands. By analysing these bands it was possible to trace two compounds as structurally similar as valine and leucine in the spectrum of leucylvaline. The result is seen in Fig. 5. The specific bands of leucine can be distinguished at 8.06μ , 8.41μ , 13.00μ , and of valine at 7.88μ , 8.48μ , 12.88μ . These respective bands occur in the spectrum of leucylvaline at 8.04μ , 8.40μ , 12.96μ and 7.89μ , 8.52μ , 12.84μ . The difference between the spectra of leucine and of valine appears most clearly at the absorption bands in the 8μ region. The result must be regarded as satisfactory, when one considers, that the difference between the valine and leucine molecules consists only of an extension of the carbon chain with a >CH_2 group. For this reason no great differences are to be expected in their absorption spectra.

Besides the absorption bands characteristic of amino-acids there are bands specific for the peptide bond, —CO—NH— , in the leucylvaline spectrum. At 3.01μ (3322 cm^{-1}) a band arising from the bondstretching vibrations in >N—H occurs, and at 6.00μ (1667 cm^{-1}) a band, which is to be assigned to the bondstretching vibrations in the >CO group, is observed^{9, 16}.

SUMMARY

The apparatus and the technique for recording the infra-red absorption spectra of amino-acids in paraffin oil suspension have been described. Further the absorption bands characteristic of amino-acids between 2.9 and 4.00μ ($3450\text{—}2500 \text{ cm}^{-1}$) and those between 5.7 and 7.6μ ($1750\text{—}1320 \text{ cm}^{-1}$) have been discussed. A method for identifying leucine and valine in the spectrum of leucylvaline by analysing the bands specific for these two amino-acids between 7.5 and 15.0μ ($1330\text{—}670 \text{ cm}^{-1}$) has been demonstrated. Since other amino-acids likewise show specific absorption bands in this latter range, it is proposed that amino-acid components of peptides, other than leucylvaline, may be identified in the same way.

This study has been supported by grants from *Statens Naturvetenskapliga Forskningsråd* and *Medicinska Forskningsanslaget*.

Table 1. The infra-red absorption bands of L-histidine hydrochloride, L-arginine, L-ornithine dihydrochloride, L-leucine, L-valine, L-valine hydrochloride and DL-leucylvaline.

λ = Wavelength in μ
 ν = Frequency in cm^{-1}
 E = Extinction = $\log_{10}(I_0/I)$

L-Histidine-HCl			L-Arginine			L-Ornithine-2HCl		
λ	ν	E	λ	ν	E	λ	ν	E
2.30	4348	0.576	2.22	4505	0.576	2.13	4695	0.224
2.94	3401	0.878	2.40	4167	0.508	3.20	3125	1.128
3.27	3058	1.040	2.98	3356	0.692	3.58	2793	0.909
3.52	2841	0.844	3.17	3155	0.859	3.81	2625	0.816
3.81	2625	0.824	3.30	3030	0.765	3.99	2506	0.668
4.21	2375	0.684	3.50	2857	0.600	5.14	1946	0.196
4.88	2049	0.673	3.63	2755	0.700	5.72	1748	1.000
5.32	1880	0.465	3.86	2597	0.524	6.27	1595	0.697
6.09	1642	0.953	5.75	1739	0.520	6.44	1553	0.504
6.22	1608	1.011	5.94	1684	0.680	6.68	1497	0.792
6.35	1575	0.972	6.20	1613	0.881	7.03	1422	0.424
6.70	1493	1.068	6.45	1550	0.804	7.18	1393	0.404
7.06	1416	0.873	6.62	1511	0.648	7.44	1344	0.340
7.27	1376	0.656	7.04	1420	0.736	7.70	1299	0.244
7.52	1330	0.957	7.53	1328	0.672	8.00	1250	0.476
7.66	1305	0.780	8.00	1250	0.444	8.31	1203	0.872
7.80	1282	0.838	8.44	1185	0.532	8.40	1190	0.885
8.39	1192	0.492	8.80	1136	0.580	8.70	1149	0.656
8.56	1168	0.596	9.42	1062	0.352	8.84	1131	0.664
8.70	1149	0.644	9.67	1034	0.312	9.20	1087	0.432
8.91	1122	0.620	10.28	974	0.488	9.40	1064	0.280
9.40	1064	0.712	10.90	918	0.336	9.60	1042	0.540
10.22	988	0.372	11.31	884	0.328	9.84	1016	0.328
10.43	959	0.664	11.82	845	0.496	10.16	985	0.420
10.90	917	0.548	12.26	816	0.496	10.64	940	0.448
11.47	872	0.876	12.72	786	0.604	11.47	873	0.424
11.96	836	0.688	12.96	772	0.632	11.99	834	0.456
12.16	823	0.936	13.85	722	0.664	12.39	807	0.416
12.40	806	0.756	14.30	699	0.584	12.62	792	0.240
13.00	769	0.424	14.70	680	0.540	12.90	775	0.228
13.20	758	0.376				13.18	759	0.488
13.60	735	0.336				13.54	738	0.272
13.78	726	0.332				13.85	722	0.204
14.10	710	0.224						
14.35	696	0.420						
14.73	679	0.248						

L-Leucine			L-Valine			L-Valine-HCl		
λ	ν	E	λ	ν	E	λ	ν	E
2.35	4255	0.120	2.32	4310	0.112	2.20	4545	0.100
3.23	3096	0.873	3.23	3096	0.920	3.23	3096	1.671
3.60	2778	0.640	3.62	2762	0.728	3.60	2778	1.088
3.70	2703	0.612	3.83	2611	0.752	3.76	2660	0.913
3.81	2625	0.628	4.68	2137	0.232	3.86	2591	0.880
4.68	2137	0.240	6.22	1608	1.480	4.09	2445	0.432
6.22	1608	1.112	6.33	1580	1.760	4.99	2004	0.272
6.34	1577	1.345	6.62	1511	1.325	5.08	1969	0.340
6.62	1511	1.064	7.05	1418	0.777	5.77	1733	1.632
7.13	1403	0.920	7.17	1395	0.808	6.23	1605	0.676
7.42	1348	0.440	7.55	1325	0.712	6.35	1575	0.536
7.51	1332	0.448	7.88	1269	0.368	6.70	1493	0.922
7.60	1316	0.504	8.48	1179	0.344	7.06	1416	0.312
7.73	1294	0.480	8.77	1140	0.360	7.40	1351	0.320
8.06	1241	0.332	9.02	1109	0.224	7.82	1279	0.388
8.41	1189	0.372	9.37	1067	0.304	8.21	1218	1.556
8.80	1136	0.324	9.70	1031	0.284	8.40	1190	0.592
9.20	1087	0.156	9.90	1010	0.172	8.53	1172	0.680
9.72	1029	0.144	10.55	948	0.208	8.88	1126	0.436
9.97	1003	0.168	10.80	926	0.156	9.06	1104	0.600
10.61	942	0.200	11.10	901	0.216	9.41	1063	0.588
10.88	919	0.228	11.24	890	0.224	9.70	1031	0.580
11.80	847	0.376	11.78	849	0.176	10.29	973	0.128
11.98	835	0.300	12.08	828	0.184	10.60	943	0.192
13.00	769	0.216	12.88	777	0.304	11.39	878	0.512
14.10	709	0.088	13.30	752	0.132	11.97	835	1.569
14.90	671	0.392	13.99	715	0.356	12.50	800	0.460
			14.87	672	0.160	13.63	733	0.616
						13.90	719	0.208
						14.06	702	0.188
						14.61	685	0.160

DL-Leucylvaline

λ	ν	E	λ	ν	E	λ	ν	E
2.26	4425	0.116	6.76	1479	0.920	10.03	997	0.268
2.91	3436	0.896	7.14	1401	1.232	10.38	963	0.236
3.01	3322	0.992	7.50	1333	0.552	10.55	948	0.232
3.22	3106	1.088	7.63	1311	0.608	11.30	885	0.220
3.63	2755	0.996	7.89	1267	0.568	11.50	870	0.228
3.76	2660	1.000	8.04	1244	0.616	12.05	830	0.356
3.85	2597	0.920	8.40	1190	0.480	12.84	779	0.520
4.69	2132	0.312	8.52	1174	0.476	12.96	772	0.588
6.00	1667	1.576	8.72	1147	0.412	13.54	738	0.440
6.10	1639	1.520	8.96	1116	0.280	13.70	730	0.468
6.35	1575	1.860	9.15	1093	0.300	14.10	709	0.412
6.59	1517	1.556	9.59	1043	0.288	14.81	675	0.388

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Received October 29, 1949.