were carried out as described (*1*). The values for the binodal curve data and the tie-line data are the average of three determinations except where noted and are presented in Table I. The plait point for these systems at various temperatures was estimated as before (*2*). The composition of the plait point as a function of temperature is presented in Table II.

RESULTS

The data obtained in the water system at higher temperatures and in the deuterium oxide system complement the results of Gilbert and Humphreys (*1*), showing once again no indication of compound formation. The present study indicated that at atmospheric pressure, as suggested by Gilbert and Humphreys, there appears to be some temperature limitation for the determination of the binodal curve. In both the water system and deuterium oxide system, an upper temperature limit at ca. 67° C. is set by the start of boiling of the samples upon the addition of ethanol, and a lower temperature limit for the binodal curve is set at ca. 10.2° C. by the appearance of a solid phase (N_2H_5Cl) upon the addition of ethanol and a concomitant temperature rise in the system. Hydrazine monochloride dissolves endothermically in water.

The area under the binodal curve for the deuterium oxide systems was slightly greater than the corresponding area for the water systems at the same temperature. Judging from the trend of the binodal curves, there is no marked difference in the solubility of the salt in either water or deuterium oxide. This observation is confirmed from the experimentally determined solubilities of the salt at various temperatures.

Table II. Estimated Composition of the Plait Point

Temp., °C.	Ethanol, Wt. %	Water, Wt. %	N_2H_5Cl, Wt. %
15[a]	22.8	45.0	33.2
25[a]	38.0	40.0	22.0
45	50.0	35.0	15.0
55	53.5	33.0	13.7
65	56.0	31.0	13.0
		D_2O, Wt. %	
15	48.2	39.1	12.7
25	50.7	37.3	12.0
45	50.3	36.2	13.5
65	52.5	33.7	13.8

[a] Taken from the data of (*1*).

The plait point composition for the water system shifts towards increasing ethanol concentration with increasing temperature. The plait point composition for the deuterium oxide system shows the same general trend, although there does not seem to be as much temperature dependence.

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Numerical Values of the Absorbances of the Aromatic Amino Acids in Acid, Neutral, and Alkaline Solutions

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Absorbances of the aromatic amino acids have been determined at frequent wavelength intervals with the Cary M14 recording spectrophotometer in neutral and alkaline solutions. Difference spectra of acid and alkaline *vs.* the neutral solution were also obtained.

NUMERICAL values of the absorbances of aromatic amino acids at frequent wavelength intervals are valuable for the calculation of a spectrum corresponding to the aromatic chromophores of a protein in their free state. The choice of the free amino acids as model compounds is not the best one, because of the unknown influence of the charges present on the α-amino and the carboxyl groups, which are absent in the polypeptide chain. However, this effect may not be very large and the calculated spectra are useful as a first approximation to evaluate the spectral shifts caused by the incorporation of the chromophores into the protein fabric.

Spectra of the aromatic amino acids have been published (*1*, *3*, *5*, *7*); however, it is difficult to obtain numerical data from these, and the numerical values published are usually confined to the spectral maxima. The necessity of detailed data of this kind for the spectral study of proteins prompted their determination, and since they proved valuable, their publication appeared desirable.

MATERIALS AND METHODS

Chromatographically pure L-tyrosine, L-tryptophan, and L-phenylalanine were obtained from H. M. Chemical Co., Ltd., Santa Monica, Calif., and from Mann Research Laboratories, Inc., New York, N. Y. Fisher reagent chemical grade L-tyrosine and L-tryptophan were also used. The amino acids from all these sources were indistinguishable

from a spectroscopical point of view; therefore, all the data were pooled. Amounts of the order of 0.4 to 0.5 gram were dried in a vacuum oven at 60° C. for 24 hours and weighed. Tyrosine and tryptophan were dissolved in glass-distilled water in a 1-liter volumetric flask and phenylalanine in a 50-ml. one. The required dilutions were prepared by pipetting the stock solutions with large volume pipets (15 to 25 ml.) into suitable volumetric flasks or by diluting them with diluents pipetted with similar pipets. From each, amino acid dilutions were made in 0.1*N* HCl, 0.1*M* phosphate buffer of pH 7.1, and in 0.1*N* KOH.

A Beckman Model DU spectrophotometer, with a standard blue sensitive phototube, was used in measurements performed many years ago. These data were supplemented recently with recordings made in the Cary spectrophotometer. The two sets of data were in reasonably good agreement. Since at the present time most of the spectroscopic work concerned with full spectra is performed with the Cary instrument, only the data obtained with the latter will be presented in this paper.

The Cary Model 14 recording spectrophotometer was operated with dynode voltage control in position 1, slit control at 50. In the 230 to 320 mμ range this resulted in slit widths varying from 0.3 to 0.1 mm. According to the dispersion data of the manufacturer this corresponds to a spectral band width of 0.3 to 0.5 mμ. Since the absorption bands of the aromatic amino acids are fairly broad, these band widths seem sufficiently narrow to give the correct spectral heights. This was checked by recording the spectrum of phenylalanine, which has the sharpest bands

Table I. Molecular Absorbances of Tyrosine

Mμ	Neutral	Alkaline
230	4980	7752 ± 108
232	3449	8667 ± 38
234	1833 ± 14	9634 ± 19
236	1014 ± 43	10440 ± 20
238	571 ± 36	11000 ± 10
240	349 ± 34	11300 ± 20
240.5 ↑	...	11340 ± 30
242	252 ± 20	11230 ± 40
244	209 ± 18	10760 ± 50
245.3 ↓	202 ± 20	...
246	205 ± 17	9918 ± 78
248	218 ± 15	8734 ± 72
250	246 ± 14	7382 ± 56
252	287 ± 13	5844 ± 77
254	341 ± 14	4471 ± 55
256	401 ± 12	3360 ± 46
258	485 ± 10	2476 ± 20
260	582 ± 9	1883 ± 17
262	693 ± 13	1467 ± 7
264	821 ± 13	1204 ± 14
266	960 ± 14	1054 ± 16
268	1083 ± 13	985 ± 13
269.3 ↓	...	974 ± 8
270	1197 ± 9	979 ± 9
272	1310 ± 9	1019 ± 8
274	1394 ± 6	1094 ± 8
274.8 ↑	1405 ± 7	...
276	1367 ± 0	1206 ± 4
278	1260 ● 2	1344 ± 5
280	1197 ± 0	1507 ± 5
282	1112 ± 2	1675 ± 5
284	845 ± 8	1850 ± 6
286	506 ± 7	2024 ± 4
288	248 ± 8	2179 ± 5
290	113 ± 0	2300 ± 7
292	50 ± 1	2367 ± 5
293.2 ↑	...	2381 ± 6
294	23 ± 1	2377 ± 8
296	13 ± 0	2317 ± 10
298	8 ± 1	2195 ± 16
300	6 ± 0	2006 ± 23
302	5 ± 1	1747 ± 29
304	3 ± 0	1445 ± 27
306	2 ± 1	1107 ± 35
308	1 ± 0	800 ± 27
310	1 ± 0	547 ± 21
312		346 ± 15
314		206 ± 12
316		118 ± 9
318		67 ± 5
320		32 ± 3
322		15 ± 3
324		6 ± 2
326		1 ± 1

Table II. Molecular Absorbances of Tryptophan

Mμ	Neutral	Alkaline
230	6818	13200
232	4037 ± 60	7470
234	2772 ± 71	4354 ± 81
236	2184 ± 64	2951 ± 50
238	1904 ± 55	2282 ± 29
240	1764 ± 52	1959 ± 30
242.0 ↓	1737 ± 49	1813 ± 25
244	1772 ± 48	1773 ± 29
244.4 ↓	...	1763 ± 29
246	1869 ± 40	1792 ± 27
248	2018 ± 35	1877 ± 23
250	2217 ± 32	2013 ± 25
252	2462 ± 19	2187 ± 37
254	2760 ± 27	2410 ± 38
256	3087 ± 20	2664 ± 25
258	3422 ± 18	2953 ± 39
260	3787 ± 17	3261 ± 34
262	4142 ± 14	3586 ± 46
264	4472 ± 10	3895 ± 32
266	4777 ± 14	4212 ± 48
268	5020 ± 15	4481 ± 46
270	5220 ± 8	4742 ± 37
272	5331 ± 5	4933 ± 45
272.1 ↑	5344 ± 5	...
273.6 ↓	5329 ± 10	...
274	5341 ± 8	5025 ± 34
274.5 ~	...	5062 ± 38
276	5431 ± 8	5108 ± 39
278	5554 ± 12	5275 ± 46
279.0 ↑	5579 ± 14	...
280	5559 ± 12	5377 ± 43
280.4 ↑	...	5385 ± 34
282	5323 ± 10	5302 ± 34
284	4762 ± 11	4962 ± 42
285.8 ↓	4471 ± 6	...
286	4482 ± 11	4596 ± 22
286.8 ↓	...	4565 ± 27
287.8 ↑	4650 ± 12	...
288	4646 ± 16	4634 ± 19
288.3 ↑	...	4639 ± 28
290	3935 ± 5	4393 ± 32
292	2732 ± 5	3551 ± 46
294	1824 ± 5	2666 ± 27
296	1211 ± 10	1990 ± 24
298	797 ± 4	1472 ± 19
300	510 ± 1	1064 ± 19
302	314 ± 3	755 ± 16
304	184 ± 2	517 ± 10
306	112 ± 4	333 ± 6
308	55 ± 9	217 ± 4
310	27 ± 11	129 ± 5
312	11 ± 8	84 ± 8
314	3 ± 2	53 ± 7
316		31 ± 7
318		17 ± 4
320		8 ± 2
322		3 ± 4

of the three amino acids investigated, at various sensitivities. A 15-fold variation in slit width resulted, without any change in the spectral heights.

The wave length scale of the instrument was checked with a low pressure mercury light, as recommended by the manufacturer, and found accurate ±2 A. The slide wires were calibrated with the alkaline chromate method of Haupt (2). The average deviation from the values given by this author was +0.01 absorbance units and no correction was made for this.

The recordings were performed at 5 A. per second scanning speed with a chart speed to give a spread of 2 mμ for every division of the chart. The spectral heights were read on the chart at every 2 mμ, and at the maxima and minima, relative to a base line recorded with cuvettes filled with solvent.

Amino acid concentrations were chosen to give absorbances at the maxima ranging from 0.3 to 1.6 absorbance units. Runs with standard chromate of twice the prescribed concentration proved that the Cary instrument gives accurate results also in the absorbance range from 1.0 to 2.0. The absorbances of the solutions were low enough to avoid stray-light and fluorescence effects (4).

RESULTS AND DISCUSSION

From three to six runs were performed at various concentration levels with each solution. The molecular absorbances of each run were calculated and the data obtained under identical conditions were averaged. These data, for each of the three amino acids investigated, are listed in Tables I, II, and III. Maxima, minima, and inflection points are indicated by ↑, ↓, and ~. The tables list also the mean deviations, except in some instances at the low wavelength end of the spectrum, when the absorbances were so high that readings could be obtained only with the solutions of the lowest concentration.

In general, the mean deviation is of the order of 1% or less. The results are given to four places, although the last figure only has a slight significance given by the fact that most of the recordings at high absorbances were close to or above 1.000. However, the main reason for maintaining the fourth figure was to give a better idea of the spread of the data.

The data presented here show a high degree of reproducibility and precision, although they were obtained from recordings performed on two different instruments (serial No. 85 and 158). A collaborative study (6) of the absorption of standard chromate solutions with 13 Cary spectro-

Table III. Molecular Absorbances of Phenylalanine

Mμ	Neutral	Mμ	Alkaline
230	32.8 ± 1.5	230	161.9 ± 1.9
232	32.1 ± 1.6	232	99.2 ± 1.9
234	35.6 ± 2.1	234	70.7 ± 2.4
236	42.8 ± 2.1	236	63.3 ± 2.7
238	48.5 ± 2.3	238	62.3 ± 2.6
240	59.4 ± 2.0	240	68.9 ± 3.2
242 ~	72.2 ± 2.3	242	83.0 ± 2.8
		243 ~	85.4 ± 2.9
244	80.1 ± 2.1	244	89.0 ± 3.0
246	102.0 ± 0.6	246	108.9 ± 2.8
247.4 ↑	110.7 ± 2.2	247	120.9 ± 1.5
248	109.8 ± 1.9	248.0 ↑	126.1 ± 1.4
248.3 ↓	109.5 ± 2.0	248.7 ↓	125.1 ± 1.7
250	123.5 ± 2.6	250	132.7 ± 1.8
251	143.0 ± 2.8	251	149.3 ± 1.9
252	153.9 ± 1.0	252	167.0 ± 1.1
252.2 ↑	154.1 ± 1.0	252.9 ↑	171.5 ± 1.3
254	139.6 ± 1.0	254	166.3 ± 0.8
254.5 ↓	138.5 ± 1.4	254.9 ↓	162.8 ± 1.7
256	156.5 ± 2.2	256	168.4 ± 1.9
257.6 ↑	195.1 ± 1.5	257	188.4 ± 2.8
258	193.4 ± 1.3	258	209.1 ± 0.3
259	171.9 ± 1.0	258.2 ↑	209.6 ± 0.2
260	147.0 ± 0.6	260	184.2 ± 1.0
261.9 ↓	127.7 ± 1.5	260.7 ~	178.6 ± 0.3
262	128.1 ± 1.4	262	157.8 ± 0.9
263.7 ↑	151.5 ± 0.6	262.7 ↓	105.5 ± 1.3
264	148.7 ± 0.4	263.9 ↑	161.2 ± 1.0
265	119.8 ± 1.3	264	160.0 ± 2.1
266	91.8 ± 1.4	266	114.3 ± 1.6
266.8 ~	85.6 ± 1.5	266.5 ↓	109.7 ± 1.8
		267.7 ↑	117.7 ± 1.8
268	74.7 ± 1.0	268	115.0 ± 1.0
270	30.0 ± 1.8	270	50.2 ± 2.0
272	14.3 ± 1.0	272	18.7 ± 1.1
274	5.4 ± 0.3	274	7.4 ± 0.3
276	2.2 ± 0.4	276	2.6 ± 0.4
278	1.1 ± 0.5	278	0.7 ± 0.3
280	0.7 ± 0.3	280 ~	0.4 ± 0.2

Table IV. Alkaline Vs. Neutral Difference Spectra of Tyrosine, Tryptophan, and Phenylalanine

Mμ	Tyrosine	Tryptophan	Phenylalanine
230	3041	4135	123.9
232	5440	3213	66.0
234	7608	1621	35.4
236	9415	732 ± 35	20.7
238	10490	345 ± 23	13.9
240	11060	149 ± 21	9.9
242	11090	45 ± 15	11.1
244	10660	−40 ± 15	8.8
246	9844	−104 ± 11	9.0
248	8567	−172 ± 10	16.4
250	7205	−233 ± 9	8.3
252	5671	−298 ± 16	15.3
254	4344	−371 ± 10	25.8
256	3127	−435 ± 14	11.3
258	2142	−490 ± 19	19.1
260	1368 ± 37	−535 ± 13	37.8
262	820 ± 36	−564 ± 8	26.9
264	420 ± 25	−580 ± 10	16.1
266	125 ± 20	−573 ± 12	22.4
268	−78 ± 18	−539 ± 14	42.8
270	−225	−486 ± 12	17.4
272	−296	−394 ± 12	4.0
274	−299	−308 ± 7	1.7
276	−158	−312 ± 16	0.3
278	89 ± 5	−278 ± 13	0
280	315 ± 1	−191 ± 18	
282	558 ± 1	−24 ± 4	
284	994 ± 10	194 ± 3	
286	1513 ± 11	110 ± 10	
288	1936 ± 1	11 ± 3	
290	2196 ± 14	467 ± 8	
292	2331 ± 15	802 ± 3	
294	2357 ± 7	830 ± 8	
296	2307 ± 6	755 ± 10	
298	2194 ± 7	652 ± 13	
300	2002 ± 3	527 ± 5	
302	1754 ± 2	413 ± 11	
304	1437 ± 2	300 ± 14	
306	1097 ± 9	205 ± 8	
308	792 ± 14	137 ± 5	
310	526 ± 13	88 ± 3	
312	334 ± 9	55 ± 8	
314	221 ± 19	22 ± 14	
316	101 ± 7	16 ± 10	
318	62 ± 2	5 ± 2	
320	28 ± 4	0	
322	12 ± 5		
324	3 ± 4		
326	1 ± 1		
328	0		

Table V. Acid *Vs.* Neutral Difference Spectra of Tyrosine, Tryptophan, and Phenylalanine

Mμ	Tyrosine	Tryptophan	Phenylalanine
230	...	... ...	46.7
232	576	421 ± 49	34.1
234	441	610 ± 37	23.3
236	346	590 ± 31	16.0
238	218	512 ± 29	10.8
240	108	432 ± 23	6.9
242	40	358 ± 19	3.9
244	4	305 ± 20	3.3
246	−13	263 ± 21	1.2
248	−18	240 ± 16	−0.3
250	−20	223 ± 14	2.5
252	−16	216 ± 17	−1.8
254	−12	219 ± 15	−1.9
256	−7	222 ± 11	4.0
258	−5	223 ± 14	−3.3
260	−3	225 ± 14	−4.3
262	0	232 ± 13	2.5
264	0	232 ± 18	−3.1
266	−4	224 ± 15	−3.4
268	−8	214 ± 7	−4.4
270	−11	190 ± 9	−2.0
272	−13	159 ± 12	−0.7
274	−20	127 ± 15	−0.3
276	−40	128 ± 16	0
278	−45	110 ± 10	
280	−34	71 ± 11	
282	−46	−23 ± 7	
284	−73	−92 ± 9	
286	−71	−3 ± 9	
288	−49	−26 ± 4	
290	−31	−250 ± 5	
292	−20	−317 ± 9	
294	−16	−276 ± 9	
296	−14	−227 ± 5	
298	−12	−177 ± 7	
300	−10	−131 ± 9	
302	−9	−88 ± 6	
304	−7	−59 ± 8	
306	−6	−40 ± 10	
308	−4	−24 ± 10	
310	−3	−13 ± 10	
312	−2	−7 ± 7	
314	−1	−4 ± 4	
316	−1	0	
318	0		

photometers demonstrated also the accuracy of this instrument. Apparently, with proper checking of the performance, the results with different instruments should agree to within 1% over most, but certainly at the upper half, of the absorbancy scale.

Difference spectra are becoming increasingly important. The alkaline-neutral difference spectrum of the three amino acids investigated can be calculated easily from the data presented. As a check on these, direct difference spectra were also recorded and the data are listed in Table IV. These figures are either from one single determination or are averages obtained from two individual runs. The calculated and the direct differences agree much better than the general 1% accuracy assumed for the spectral data.

More attention was paid in this work to neutral and alkaline solutions, because these are of general applicability to proteins. In acid solution, a large number of the latter are insoluble. Nevertheless, to have a complete set of data, difference spectra of acid-neutral solutions of the aromatic amino acids were also run and the data are presented in Table V. The tyrosine and phenylalanine data are from a single run, those for tryptophan are the averages of two runs. Apparently, the differences are much smaller than with the alkaline-neutral pairs. The spectra of the three amino acids in acid solution can be calculated from the neutral and the corresponding acid difference spectra.

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Correlation Studies on Vapor Pressures and Critical Properties for Isomeric Alkanes

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IN SPITE of the great amount of effort that has been expended in developing correlation procedures for physical properties of branched alkanes, the authors still find the situation in a somewhat unsatisfactory state. Furthermore, recently additional, precise data have become available (*1, 12*), which serve as an incentive to re-examine the status of the problem on branched alkanes, with the further goal in mind that these hydrocarbons may serve as a framework for properties of alkyl derivatives. Thus, the authors have chosen to examine critically the vapor pressure and boiling point data and the values of the critical points of isomeric alkanes from the butanes (C_4) through the decanes (C_{10}).

Structural dependence of isomeric variations in physical and thermodynamic properties has been the subject of investigation by various authors (*5, 6, 11, 15, 18*). Greenshields and Rossini (*6*) have correlated the variation in physical and thermodynamic properties between an isomer and the normal compound with the structural param-