nation of corrections for heat interchange by controlling the temperature of a shield becomes very difficult. In the temperature range above 250°K., on the other hand, where a difference thermocouple is very sensitive and where the corrections for thermal relaxation times within the calorimetric system become important, we believe that an adiabatic calorimeter is capable of more accurate results than our isothermal calorimeter. Even in this region, however, a comparison of our measurements of the heat capacity of benzoic acid with those of the other laboratories indicates that our estimate of a possible error of 0.4% at 300°K. is a generous one.

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[CONTRIBUTION FROM THE DEPARTMENTS OF CHEMISTRY AND PHYSIOLOGY AND THE INSTITUTE FOR THE STUDY OF METALS, UNIVERSITY OF CHICAGO, CHICAGO, ILLINOIS]

Heat Capacities from 11 to 305°K. and Entropies of *l*-Alanine and Glycine[1]

BY JOHN O. HUTCHENS, ARTHUR G. COLE AND J. W. STOUT

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The heat capacities of *l*-alanine and glycine have been measured from 11 to 305°K. The entropies at 298.15°K. were found to be 30.88 cal. deg.$^{-1}$ mole^{-1} for *l*-alanine and 24.74 cal. deg.$^{-1}$ mole^{-1} for glycine. The heat capacities, entropies, $(H^\circ - H^\circ_0)/T$ and $-(F^\circ - H^\circ_0)/T$ are tabulated for both substances from 10 to 310°K. The values of $S^\circ_{298.15} - S^\circ_{90}$ found are in good agreement with previous work by Huffman and Borsook on *d*-alanine and by Parks, *et al.*, on glycine. However, extrapolation procedures employed below 90°K. by previous workers led to too high an estimate for S°_{90} for both amino acids.

One of the most obvious deficiencies in present day knowledge of the metabolism of living organisms is the lack of information concerning energetic changes associated with the synthesis of proteins. For several years we have been engaged in measuring the heat capacities of the naturally occurring amino acids and some selected proteins to establish the entropies of these compounds. Concurrently Tsuzuki and Hunt[2] have been measuring the heats of combustion of these same compounds. The projects were undertaken to supplement work on the few amino acids studied in the 1930's by Huffman, Borsook and others.[3]

We present here our data on glycine and *l*-alanine. *d*-alanine has been studied previously[4] as has glycine[5] but not below 90°K. Estimates of the entropies of these amino acids must be revised as a result of extending heat capacity measurements below 90°K. Furthermore, over the entire range of temperatures employed, our data offer a greater degree of precision than the previous studies.

Experimental

Amino Acids.—The glycine and *l*-alanine used in these studies were provided by the late Jesse P. Greenstein of the National Institutes of Health. Both were free of other amino acids as indicated by unidirectional paper chromatography using five different solvent systems (formix, MeOH–pyridine, ketone mix, phenol and butanol–acetic acid). The *l*-alanine was 99.9% free of the *d*-isomer by the criteria of Meister, *et al.*[6] Both amino acids were crystalline as evidenced by examination in polarized light. Both materials were dried at room temperature for several days at 10^{-4} mm. in a system trapped with liquid nitrogen. After loading the calorimeter they were subjected to pumping at $<10^{-6}$ mm. pressure for 48 hr. No weight losses which could be considered significant occurred. Nonetheless the heat capacity measurement on glycine at 269.27°K. is 1.1% high and was ignored in drawing the curve from which the thermodynamic functions were derived. We believe the high value probably arises from the melting of water in the sample. To produce the excess heat observed in this measurement, 0.022 g. H_2O per mole of glycine would be required. At other temperatures the effect on the heat capacity of this amount of water is always less than 0.06%, and since it is small compared to the experimental error, no correction has been made.

Experimental Methods and Calculations.—The methods for measuring heat capacities and for calculating the results have been described in detail.[7] As an additional detail, the method used to extrapolate the heat capacity curve below 11 °K. was as follows. Values of $(\Delta H/\Delta T)/T^2$ were plotted as a function of T and this curve extrapolated in such a fashion that $\Delta H/\Delta T$ was proportional to T^3 as T approached zero. The fractional error involved is no doubt appreciable, but since S°_{10} is less than 0.2% of $S^\circ_{298.15}$ the absolute error is tolerable. The calorimeter used was the one with six radial fins and the heater-thermometer was the one bearing the laboratory designation "H" described in the previous paper. The sample weight was 47.431 g. for *l*-alanine and 98.129 g. for glycine. The heat capacity of the empty calorimeter represented 40–50% of the total heat capacity over most of the temperature range employed, falling to 25% at 300°K. in the case of glycine.

Results

The experimentally measured quantities ($\Delta H/\Delta T$) are given in Table I for glycine and in Table II for *l*-alanine. The individual values are listed in the chronological order in which they were obtained and the thermal history of the compounds can be deduced from the table. Glycine had an exceptionally stormy history as a result of a broken vacuum line. In general the temperature rise in individual experiments can be deduced from the intervals between the points. Where an occasional run was lost or where cooling overnight led to overlapping of points, the following applies: From 11–50°K. ΔT was approximately 0.1 T. From 50–150°K., ΔT <5.5°, from 150–200°K., ΔT <6°;

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TABLE I

HEAT CAPACITY OF GLYCINE IN CAL. DEG.$^{-1}$ MOLE^{-1}

0°C. = 273.15°K. Mol. wt. $CH_2NH_2CO_2H$ = 75.07

T, °K.	ΔH/ΔT	T, °K.	ΔH/ΔT	T, °K.	ΔH/ΔT
301.15	23.94	Run lost		241.76	19.91
301.70	23.91	89.92	9.441	299.09	23.70
53.74	5.137	95.24	9.920	231.32	19.21
58.91	5.861	100.31	10.36	238.22	19.66
64.10	6.556	105.20	10.77	244.97	20.12
68.83	7.144	108.88	11.07	252.08	20.60
73.88	7.728	114.31	11.51	261.61	21.24
78.68	8.266	119.92	11.94	269.27	22.01
83.84	8.835	125.09	12.32	276.69	22.27
88.55	9.314	130.32	12.69	284.28	22.78
93.58	9.781	134.32	13.01	292.23	23.28
98.65	10.22	139.73	13.39	11.58	0.096
103.55	10.62	145.14	13.74	12.40	.126
109.46	11.13	150.57	14.12	13.68	.170
115.19	11.57	156.04	14.48	15.15	.241
188 → 300 →		161.50	14.84	16.84	.335
112°K.		165.73	15.10	18.63	.462
114.28	11.52	171.66	15.49	20.64	.627
119.35	11.89	177.30	15.87	22.54	.804
124.30	12.26	183.07	16.22	24.81	1.048
124.45	12.28	188.84	16.57	27.50	1.360
129.32	12.64	191.43	16.72	30.34	1.727
300.96	23.94	197.09	17.11	33.46	2.158
53.40	5.090	202.97	17.47	36.87	2.647
58.81	5.848	208.91	17.83	40.54	3.188
64.10	6.556	214.95	18.20	44.65	3.802
69.82	7.267	220.90	18.56	49.19	4.482
74.59	7.825	227.58	19.02		
79.33	8.349	234.57	19.44		

from 200–250°K., ΔT <6.5°; from 250–310°K., ΔT <7°. Analysis of the individual experiments indicated that no curvature corrections were necessary within the limits of accuracy claimed[7] except for the point at 11.58°K. (Table I) where the correction is −0.7%. With this exception the values of $\Delta H/\Delta T$ given in Table I are equal to C_p at the given temperatures. The ice point was taken as 273.15°K. and 1 cal. = 4.1840 abs. joules.

TABLE II

HEAT CAPACITY OF *l*-ALANINE IN CAL. DEG.$^{-1}$ MOLE^{-1}

0°C. = 273.15° K. Mol wt. $CH_3CHNH_2CO_2H$ = 89.09

T, °K.	ΔH/ΔT	T, °K.	ΔH/ΔT	T, °K.	ΔH/ΔT
302.25	29.67	161.65	18.38	305.40	29.75
56.13	6.820	167.87	18.91	300 → 10 →	
61.46	7.637	174.16	19.44	300°K.	
67.20	8.462	180.11	19.90	300.96	29.28
		186.25	20.38	11.66	0.186
Run lost		192.33	20.87	12.44	.223
77.60	9.852	198.59	21.34	13.74	.303
82.74	10.56	205.58	21.90	15.22	.422
87.68	11.18	212.68	22.44	16.94	.582
92.94	11.78	219.55	22.93	18.73	.771
98.37	12.37	225.60	23.45	20.69	1.004
104.03	12.99	232.38	23.99	23.27	1.358
109.82	13.60	239.42	24.58	25.89	1.744
115.70	14.17	246.51	25.25	28.61	2.182
120.65	14.68	253.46	25.71	31.63	2.691
126.48	15.22	260.50	26.21	34.91	3.277
132.28	15.79	267.63	26.78	38.49	3.896
138.06	16.34	273.80	27.39	42.36	4.551
143.83	16.82	280.91	27.90	46.83	5.316
149.61	17.31	288.46	28.51	51.88	6.142
155.54	17.86	296.23	29.01	57.16	6.967

The thermodynamic properties of glycine derived from the data of Table I are given in Table III and equivalent data for *l*-alanine in Table IV.

TABLE III

THERMODYNAMIC PROPERTIES OF GLYCINE, CAL. DEG.$^{-1}$ MOLE^{-1}

T, °K.	$C_p°$	$S°$	$\frac{H° - H°_0}{T}$	$\frac{-(F° - H°_0)}{T}$
10	0.061	0.019	0.015	0.005
15	.231	.071	.054	.017
20	.572	.180	.138	.043
25	1.067	.328	.247	.082
30	1.682	.576	.433	.142
35	2.374	.886	.660	.226
40	3.108	1.251	.920	.331
45	3.853	1.660	1.205	.455
50	4.600	2.105	1.507	.598
55	5.321	2.577	1.821	.756
60	6.012	3.070	2.142	.928
70	7.287	4.095	2.788	1.307
80	8.427	5.144	3.423	1.721
90	9.449	6.197	4.037	2.160
100	10.34	7.239	4.623	2.616
110	11.16	8.263	5.180	3.083
120	11.95	9.268	5.712	3.556
130	12.69	10.25	6.221	4.034
140	13.40	11.22	6.709	4.513
150	14.08	12.17	7.178	4.992
160	14.74	13.10	7.630	5.469
170	15.39	14.01	8.067	5.945
180	16.02	14.91	8.492	6.418
190	16.65	15.79	8.904	6.889
200	17.28	16.66	9.307	7.356
210	17.90	17.52	9.702	7.819
220	18.52	18.37	10.09	8.280
230	19.14	19.20	10.47	8.736
240	19.80	20.03	10.84	9.190
250	20.46	20.85	11.21	9.640
260	21.14	21.67	11.58	10.09
270	21.81	22.48	11.95	10.53
280	22.48	23.29	12.31	10.97
290	23.16	24.09	12.68	11.41
300	23.84	24.88	13.04	11.85
310	24.53	25.68	13.40	12.28
273.15	22.02	22.73	12.06	10.67
298.15	23.71	24.74	12.97	11.77
310.15	24.54	25.69	13.40	12.29

Discussion

Over the temperature range in which previous studies were made, and to the degree of accuracy claimed[4,5] we find good agreement. Thus Parks, *et al.*,[5] give $S°_{298.15} - S°_{90}$ for glycine as 18.57 cal. deg.$^{-1}$ mole^{-1}; we find 18.54 cal. deg.$^{-1}$ mole^{-1}. Huffman and Borsook[4] give $S°_{298.15} - S°_{90}$ as 22.77 cal. deg.$^{-1}$ mole^{-1} for *d*-alanine; we find 22.88 cal. deg.$^{-1}$ mole^{-1} for *l*-alanine. Thus the differences between our entropy values and theirs at the various temperatures are to be attributed largely to the extrapolation procedures used by the previous workers below 90°K. This is of considerable importance since $S°_{90}$ in both cases represents *ca.* 25% of $S°_{298.15}$. Their value of $S°_{90}$ = 8.88 cal. deg.$^{-1}$ mole^{-1} for *d*-alanine differs by 0.88 cal. deg.$^{-1}$ mole^{-1} from

TABLE IV

THERMODYNAMIC PROPERTIES OF *l*-ALANINE, CAL. DEG.$^{-1}$ MOLE^{-1}

T, °K.	$C_p°$	$S°$	$\frac{H° - H°_0}{T}$	$\frac{-(F° - H°_0)}{T}$
10	0.118	0.040	0.030	0.010
15	.400	.133	.100	.033
20	.920	.314	.236	.079
25	1.607	.591	.439	.152
30	2.412	.954	.699	.255
35	3.286	1.391	1.006	.385
40	4.151	1.887	1.346	.541
45	4.997	2.425	1.704	.720
50	5.832	2.995	2.076	.919
55	6.640	3.589	2.454	1.135
60	7.414	4.200	2.836	1.364
70	8.850	5.452	3.594	1.859
80	10.19	6.722	4.335	2.387
90	11.44	7.996	5.056	2.940
100	12.56	9.260	5.752	3.509
110	13.61	10.51	6.419	4.088
120	14.60	11.73	7.059	4.674
130	15.56	12.94	7.677	5.264
140	16.49	14.13	8.274	5.855
150	17.38	15.30	8.851	6.445
160	18.24	16.45	9.411	7.035
170	19.08	17.58	9.956	7.622
180	19.89	18.69	10.49	8.206
190	20.68	19.79	11.00	8.786
200	21.46	20.87	11.50	9.364
210	22.23	21.93	12.00	9.937
220	23.00	22.98	12.48	10.51
230	23.80	24.02	12.95	11.07
240	24.60	25.05	13.42	11.63
250	25.41	26.07	13.89	12.19
260	26.22	27.09	14.34	12.74
270	27.02	28.09	14.80	13.29
280	27.82	29.09	15.25	13.84
290	28.60	30.08	15.70	14.38
300	29.36	31.06	16.14	14.92
310	30.11	32.04	16.58	15.46
273.15	27.27	28.41	14.94	13.46
298.15	29.22	30.88	16.06	14.82
310.15	30.12	32.05	16.58	15.47

our value of 7.996 for *l*-alanine. In the case of glycine the difference is even greater. Parks, *et al.*,[5] give $S°_{90} = 7.55$ cal. deg.$^{-1}$ mole^{-1} which differs by 1.35 cal. deg.$^{-1}$ mole^{-1} from our value of 6.197 cal. deg.$^{-1}$ mole^{-1}. In both cases it will be seen that the extrapolation procedure led to too high a value for $S°_{90}$. As will be seen from Tables III and IV $S°_{10}$ amounts to only 0.02 cal. deg.$^{-1}$ mole^{-1} for glycine and 0.04 cal. deg.$^{-1}$ mole^{-1} for *l*-alanine, in each case $<0.2\%$ of $S°_{298}$.

We have rejected the temptation to recalculate the free energy changes associated with formation of peptide bonds involving glycine and *l*-alanine. More reliable data on the entropies of peptides as well as more precise heats of combustion on all compounds involved will be required before existing uncertainties[8] can be resolved. Nevertheless it is clear that, for example, in the formation of glycylglycine the $T\Delta S$ term in the equation for the free energy would be affected to the extent of *ca.* 800 cal. by our values for $S°_{298.15}$ for glycine. This is considerable in a reaction which on the basis of older figures involves only some 3500 cal. at 298°K.

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The Preparation of $(Nb_6Cl_{12})Cl_2 \cdot 7H_2O$[1]

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$Nb_6Cl_{14} \cdot 7H_2O$ was first prepared in 1913 by reduction of $NbCl_5$ with sodium amalgam, but the yield was not high and some recent attempts to use the method for large-scale preparations have been disappointing. An investigation has been made of the use of other metals as reducing agents, of which cadmium was found to be the most satisfactory. Essential details of a new procedure for preparing $Nb_6Cl_{14} \cdot 7H_2O$ from metallic niobium are given.

The complex chloride of niobium $Nb_6Cl_{14} \cdot 7H_2O$ was first prepared in 1913[3]; its properties were shown to correspond to the formula $(Nb_6Cl_{12})Cl_2 \cdot 7H_2O$. X-Ray diffraction studies of concentrated solutions[4] showed the configuration of the $Nb_6Cl_{12}^{++}$ ion to be an octahedral grouping of niobium atoms with chlorine atoms on the radial perpendicular bisectors of the edges of the octahedron. New interest in the complex ions $Nb_6Cl_{12}^{++}$ and $Ta_6Cl_{12}^{++}$ has been aroused by their proposed use for the growth of isomorphous protein crystals suitable for X-ray analysis.[5] Attempts were made in this Institute to prepare the compounds $(Ta_6Cl_{12})Cl_2 \cdot 7H_2O$ and $(Nb_6Cl_{12})Cl_2 \cdot 7H_2O$ for incorporation into protein crystals. The former compound was prepared without difficulty by the method described by Lindner and Feit.[6] Several attempts in these Laboratories by other investigators to repeat the preparation of the corresponding niobium compound were unsuccessful. An investigation of the preparation of this compound therefore was undertaken by us.

First, the preparation of $Nb_6Cl_{14} \cdot 7H_2O$ by the procedure originally used by Harned[3] was repeated. It was successful except for the low yield (about 1%) relative to the amount of $NbCl_5$ used. In an

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