(4) N_2H_4 (liq.) + $279H_2O$ = $(N_2H_4{\cdot}H_2O){\cdot}278H_2O$ $\Delta H_{298} = -3890$

one obtains

(5) N_2H_4 (liq.) + ∞H_2O = $(N_2H_4{\cdot}\infty H_2O)$ (liq.) $\Delta H_{298} = -3895$

From Eqs. (1) and (5) then may be obtained the desired result

(6) N_2H_4 (liq.) + H_2O (liq.) = $N_2H_4{\cdot}H_2O$ (liq.) $\Delta H_{298} = -1797$ cal.

This work has been made possible by grants from the National Research Council, and the General Research Council of this College.

Summary

1. Specific heats for aqueous solutions of hydrazine hydrate are reported.

2. Heats of solution for the hydrate and anhydrous hydrazine have been determined.

3. The heat of hydration of anhydrous hydrazine has been calculated.

CORVALLIS, OREGON RECEIVED JUNE 23, 1937

[CONTRIBUTION FROM THE WILLIAM G. KERCKHOFF LABORATORIES OF THE BIOLOGICAL SCIENCES OF THE CALIFORNIA INSTITUTE OF TECHNOLOGY]

Thermal Data. VII. The Heats of Combustion of Seven Amino Acids

BY HUGH M. HUFFMAN, SIDNEY W. FOX AND EMORY L. ELLIS

In continuation of our program of determining thermal data on organic compounds of physiological importance, we present in this paper the heats of combustion of seven amino acids. In two cases these data enable us to compare the heat content of the optically active with that of the inactive form.

Calorimetric Method

The calorimetric method, apparatus, units and corrections used have been described in previous publications.[1] No essential changes in method or apparatus have been made. All of the combustions were made in a Parr bomb having a volume of 0.380 liter, and with an initial oxygen pressure of 30 atmospheres and with 1 ml. of water in the bomb. The platinum wire technique[1a] was used exclusively. The heat capacity of our calorimeter has been controlled carefully throughout the course of this investigation by numerous calibrations with Bureau of Standards benzoic acid standard samples 39d and 39e, having for its isothermal heat of combustion at 25.0° the value of 26,419 international joules[2] per gram true mass. As previously reported we have used oxygen from a number of cylinders supplied by the Linde Air Products Company and at varying cylinder pressures. We have continued to obtain the same high precision in our calibrations, namely, an extreme deviation from the mean of slightly more than 0.01%. Up to the present time we have been unable to detect any effect, as has been reported by Keffler,[3] due to change in the oxygen pressure in the cylinder or in changing from cylinder to cylinder.

Units and Corrections

The unit of energy used throughout this paper is the defined conventional calorie which is derived from the international joule by multiplying by the factor 1.0004/4.185. The method of calculation, the corrections and the symbols used are those given by Stiehler and Huffman.[1b] The molecular weights are based on the 1937 table of atomic weights. This involves one important change, namely, the change in the atomic weight of carbon to 12.01. This should be borne in mind when utilizing these data in conjunction with earlier values. In applying the correction for true mass we have used approximate values of the densities obtained from the mass and volume of the pelleted material when other data were not available in the literature. All of the thermal data are given for the isothermal process at 25.0°.

In some cases the combustion was incomplete leaving a small residue of carbon. In other cases there was a residue of ash in the crucible. We have corrected for these in the manner previously reported.[1c] The magnitude of these corrections will be discussed further under the section on preparations, etc.

Purity and Dryness of the Materials Used

We have followed the general method of Stiehler and Huffman[1b] to assure ourselves of the pur-

---

[1] (a) Huffman and Ellis, THIS JOURNAL, **57**, 41 (1935); (b) Stiehler and Huffman, *ibid.*, **57**, 1734 (1935); (c) Huffman, Ellis and Fox, *ibid.*, **58**, 1728 (1936).

[2] Jessup and Green, *Bur. Standards J. Research*, **10**, 552 (1933).

[3] (a) Keffler, THIS JOURNAL, **56**, 1259 (1934); (b) Keffler, *J. Phys. Chem.*, **39**, 277 (1935); (c) Keffler, *J. chim. phys.*, **32**, 91 (1935).

ity and dryness of the combustion samples. We have made routine ash determinations on each combustion sample. We have also made suitable analyses, such as determination of the nitrogen content, titrations, and measurements of the optical rotation. We have attempted, furthermore, to define accurately the physical state of the compounds by photomicrographs of their crystalline condition as well as by a precise description of the method of preparation.

Preparation, Purification, and Heats of Combustion of the Compounds

***dl*-Alanine.**—Materials from four commercial and one unknown source were utilized in preparing sixteen different combustion samples.

(a) Material from an unknown source was twice precipitated from an aqueous solution by the addition of twice the volume of 95% ethanol. The fraction thus obtained was twice recrystallized by dissolving in boiling water and allowing the solution to cool.

(b) A Hoffmann–La Roche product which had been purified extensively for heat capacity measurements was subjected to three additional crystallizations from water.

(c) A product obtained from the "Amino Acid Manufacturers" was dissolved in boiling water and allowed to crystallize in the cold room. The mother liquor was treated with two volumes of 95% ethanol. The two fractions thus obtained were combined and were again dissolved in boiling water and allowed to crystallize.

(d) The solute in the mother liquor from (c) was precipitated with two volumes of 95% ethanol.

(e) A portion of (d) was twice recrystallized from water and finally from hot water by the addition of alcohol.

(f) A portion of (e) was dissolved in hot water and allowed to cool. After standing for several days the mother liquor was filtered off and treated with ethanol. The precipitate thus obtained was twice recrystallized from water.

(g) The mother liquors from (c) and (f) were combined and ethanol added. The precipitate thus obtained was twice crystallized from water.

(h) A sample made up of accumulated residues was benzoylated with benzoyl chloride, in chilled aqueous solution, in the presence of an excess of sodium bicarbonate. The benzoylalanine, obtained by pouring the reaction mixture into concentrated hydrochloric acid, was washed with water and boiling carbon tetrachloride and finally twice recrystallized from water. A portion of this benzoylalanine was refluxed with 48% hydrobromic acid. The hydrobromic acid was removed by vacuum distillation on the water-bath, followed by three additions of water and its subsequent removal by vacuum distillation. The residue was neutralized to litmus with ammonium hydroxide and again evaporated. The residue thus obtained was washed free of halides with absolute ethanol and finally crystallized from water.

(i) Duplicate of (c).

(j) Duplicate of (d).

(k) Some alanine residues were twice recrystallized from water by the addition of methanol.

(l) A sample of Pfanstiehl α-alanine was decolorized with norite and crystallized three times from water.

(m) A sample of Eastman *dl*-alanine was decolorized with norite and twice recrystallized from water.

(n) The mother liquor from (m) was diluted to twice its original volume and the alanine precipitated by the addition of two volumes of dioxane. The precipitate was dissolved in cold water and the alanine precipitated by the addition of 2.5 volumes of alcohol followed by 2.5 volumes of dioxane.

(o) Alanine residues were recrystallized four times from seven volumes of a 50% pyridine–water mixture and the crystals finally washed with boiling absolute alcohol.

(p) Alanine residues were twice crystallized from 3 volumes of 5% acetic acid. The crystals were finally washed with boiling absolute ethanol.

Nitrogen determinations on sample (h) gave the theoretical results. Tests with the polarimeter showed this material to be optically inert. In our early preparations it was found that the ash content was high and for this reason we have attempted to obtain an ash-free substance by resorting to crystallizing from various solvents and under various conditions. While we were able to lower the ash content we were never able to obtain a sample which was completely ash-free. The average ash content of the preparations which yielded acceptable combustion values was 0.016%, and in the extreme cases 0.04%. As has been our practice in the past we have arbitrarily corrected the mass of the substance burned by subtracting the amount of the ash found. Where the ash varied this practice always brought the values into better agreement.

Combustions were made on samples of (a) and (b) which had been dried in phosphorus pentoxide vacuum. These values were low and later determinations showed that this probably was due to incomplete removal of water. We were unable to check this point on these particular samples because of lack of material.

The combustions of samples (c) and (d) also led to low results, which could not be due to moisture content, as samples dried for three days and nine days at 100° gave values which were in excellent agreement among themselves. However, a sample dried for sixty days in phosphorus pentoxide vacuum gave a value nearly 15 calories lower. This difference was just accounted for by the loss of mass of samples whose history was the same except for additional drying at 100°.

Further purification of samples (c) and (d) led to an increase in their combustion values which were then in excellent agreement with preparations from other sources which yielded acceptable values.

There is, of course, the possibility that the low values of (c) and (d) may be due to a difference in crystal form. For this reason an attempt was made to duplicate these preparations, samples (i) and (j), one successful combustion on (i) was in good agreement with the earlier combustions. Since some of the other preparations were finally crystallized under the same conditions and since they had the same appearance under the microscope we have concluded that these low results were due to impurities.

The combustions on this material were further complicated by the fact that it is often projected from the crucible when partially burned as was the case for *d*-alanine.[1c] For this reason and on account of the small amount of some of the samples available we were unable to obtain data on all of the preparations. We were able, however, to obtain consistent data on six different preparations.

An auxiliary substance, oil, was used in all of the combustions in an attempt to decrease the number of combustions in which spattering occurred. This also tends to decrease the amount of water sorbed by the sample and it is very probable that the heat effect is quite small. In fact data obtained on preparation (c) gave the same result with or without oil.

***d*-Arginine.**—Two commercial products and several natural sources were utilized to prepare eleven different combustion samples.

Fig. 1.

(a) A material obtained from Hoffmann–La Roche was subjected to three crystallizations from carbon dioxide-free water in an apparatus designed to exclude carbon dioxide.

(b) Hoffmann–La Roche arginine which had been purified for heat capacity measurements was twice crystallized from carbon dioxide-free water.

(c) A portion of the same starting material as in (a) was twice crystallized from carbon dioxide-free water.

(d) A student preparation from sardine sperm; this was found to be largely inorganic.

(e) The unused portions of (a), (b), (c) were combined and subjected to two crystallizations from carbon dioxide-free water and with minimum exposure to carbon dioxide of the air.

(f) Argininium chloride was prepared from commercial gelatin by the flavianate method of Brand and Sandberg.[4] The hydrochloride thus obtained was decolorized with norite in a solution about one normal in respect to hydrochloric acid. The free base was obtained by adding a 5% excess of freshly prepared and washed silver oxide to an aqueous solution of the hydrochloride and stirring for fifteen minutes. The filtrate from the above was saturated with hydrogen sulfide, filtered and treated with norite. This solution was filtered and evaporated to give crystals of arginine, which then were twice crystallized from water.

(g) A portion of the crystals from the first aqueous crystallization of the above preparation was twice crystallized from the minimum amount of hot water by the addition of two and one-half volumes of carbon dioxide-free absolute ethanol.

(h, i, j, k) In addition to the above preparations samples were prepared from a crude arginine flavianate obtained from hogs' blood, from casein by the procedure used in preparation (f), from sardine sperm, and from arginine hydrochloride obtained from Pfanstiehl. Considerable difficulty was experienced in purifying the arginine from these sources and in no case were we able to obtain a preparation of satisfactory purity.

Kjeldahl nitrogen determinations on preparations (a) and (b) gave 31.48 and 31.39, respectively; theoretical, 32.16. The optical rotation of samples (e) as determined in a 5% hydrochloric acid solution with a ratio of 10.3 moles hydrochloric acid to 1 mole of arginine gave $[\alpha]^{25}\text{D}$ +28.8°.

These samples contained a small amount of ash which was usually less than 0.01%. This was corrected for in the usual manner. Occasionally small amounts of carbon were produced; this was corrected for by determining the loss in weight of the crucible after drying on the hot plate and igniting. In this connection a phenomenon which we have observed might be worth mentioning. This is, if the ash from successive combustions is allowed to accumulate in the crucible it has a tendency, if basic in character, to combine with the acids produced in the combustion with the production of salts which are decomposed on ignition, hence leading to an enormously high value for carbon. We have noticed this loss in weight many times when there was not the slightest evidence of carbon in the crucible.

Our early experiments on samples (a) and (b) indicated that this material could be dried completely in either phosphorus pentoxide vacuum or *in vacuo* at 70°. Experiments on samples (f) and (g) did not lead to the same conclusions. Our first combustions on the latter gave low results when dried in phosphorus pentoxide vacuum and in the vacuum oven; we have consequently resorted to heating at 105° in the air. We have carefully checked the change in mass of samples so exposed and find that they soon reach a steady state, thus indicating that they are not taking up carbon dioxide from the air. It is conceivable that this difference in the tendency of the various samples to cling to water is due to a difference in physical state or to the presence of small amounts of impurities or both.

Arginine after careful drying shows a tendency to increase rapidly in weight when exposed to water vapor. Under the conditions of a regular combustion a sample was found to have gained about 10 mg. We have checked the effect of the sorption of water by the sample in two ways, first, by burning a sample which had been completely covered with oil, and second by burning several samples without adding water to the bomb. The first experiment was done on sample (f) and agreed exactly with other combustions in that group. This sample, however, gave a combustion value of about 2.5 calories lower than the value

---

[4] *Org. Syntheses*, **12**, 4 (1932).

we have accepted and consequently has not been included in the table. The experiments without water in the bomb were done on sample (a) and were in good agreement with the accepted value. However, since the oxygen used was not completely dry there is some uncertainty in the correction for the vaporization of the water and we have not included these values. We feel that these experiments do show that the water sorbed by the sample does not affect the combustion values appreciably.

Samples h, i, j, and k either gave low values or were not burned. All of these samples showed marked evidence of charring when heated at 100°. This was not true of samples e, f, and g, and evidently was due to impurities in the samples.

Glycine.—Material from three commercial sources was utilized to prepare five combustion samples.

(a) A Pfanstiehl product was once crystallized from water.

(b) A Hoffmann–La Roche product was crystallized from water three times and finally from water by the addition of ethanol.

(c) Material from I. Cornet was crystallized once from water.

(d) An aqueous solution of the same starting material as in (b) was boiled twice with norite, filtered hot and precipitated with ethanol. This was followed by one crystallization from water and finally by dissolving in water and precipitating by the addition of ethanol.

(e) A portion of preparation (a) was electrodialyzed for seven hours. The solution from the central chamber of the apparatus was treated with ethanol.

Nitrogen determinations gave the theoretical results. There were small amounts of ash in all of these samples, which amounted in all cases to less than 0.01%. There were usually small amounts of carbon in the crucible. The correction for this was of the order of 0.02%.

In addition to the data given in Table I five additional combustions were made on samples of (a), (b), (c), and (d) which were dried in phosphorus pentoxide vacuum. These led to a value for the heat of combustion which was 0.04% low. It was found that additional mass was lost by drying at 100° to account for this difference in combustion value.

***dl*-Leucine.**—Materials from two different sources were utilized in preparing two combustion samples.

(a) *dl*-Leucine which had been kindly supplied and purified by K. Linderstrøm-Lang for heat capacity measurements was subjected to three additional crystallizations from water by allowing the hot solutions to stand in the cold room.

(b) A commercial material supplied by the "Amino Acid Manufacturers" was once recrystallized from water as above.

Nitrogen analyses on sample (a) gave theoretical results. The ash content was of the order of 0.01% and was corrected for in the usual way. Test with the polarimeter showed this material to be optically inert. Occasionally carbon was found in the crucible, the correction for this being also about 0.01%.

***d*-Leucine.**—The residues from the *dl*-leucine preparations were utilized as the starting material. These were formulated by the method of Steiger[5] in a formulation apparatus kindly loaned to us by Professor M. S. Dunn. The resolution was made according to the method of Fischer and Warburg[6] with the following modifications in the hydrolyses. The formyl-*d*-leucine was refluxed for one hour with fifteen times its weight of 10% hydrobromic acid. The solution was evaporated *in vacuo* with the subsequent addition of water three times, neutralized to litmus with concentrated ammonium hydroxide, filtered, and the leucine washed with absolute ethanol until halide free.

(a) The *d*-leucine obtained by the above method was recrystallized once from water.

(b) The mother liquor from (a) was concentrated by pumping off a part of the water and a second crop of crystals obtained.

The nitrogen was found to be the theoretical. The rotation, determined with a solution containing 20% hydrochloric acid and with a ratio of hydrochloric acid/leucine of 25 gave $[\alpha]^{24}\text{D}$ −15.7°. The ash content was negligible and in only one case was there any evidence of carbon.

***l*-Leucine.**—Preparations from Hoffmann–La Roche, Carlsberg Laboratory and from the resolution of *dl*-leucine were the starting materials for eleven combustion samples.

(a), (b), (c), (d), (e) Numerous attempts were made by recrystallization, etc., to obtain samples of Hoffmann–La Roche *l*-leucine free from sulfur but without succeeding.

(f) Hoffmann–La Roche *l*-leucine was formulated by the Steiger[5] method. The formyl-*l*-leucine thus obtained was crystallized six times from water. It was then hydrolyzed with hydrochloric acid and neutralized with lithium hydroxide according to the method of Fischer and Warburg.[6] The crystals thus obtained were washed with ethanol.

(g) The *l*-leucine obtained in the resolution of *dl*-leucine, described under *d*-leucine, was recrystallized similarly from water.

(h) This sample was obtained by evaporating the mother liquor from (g) *in vacuo* until crystals appeared.

(i) A portion of (f) was once recrystallized from water.

(j) A collection of *l*-leucine residues was formulated by the method of Fischer and Warburg[6] for synthetic leucine. The formyl-*l*-leucine thus obtained was recrystallized from water six times. It was hydrolyzed with hydrobromic acid and neutralized with ammonium hydroxide in the manner already described. The material from the above treatment was recrystallized once from water.

The nitrogen analyses on the above samples were the theoretical. Sulfur analyses were completely negative. The rotation, determined on a solution containing 20% hydrochloric acid and with a ratio of hydrochloric acid/leucine of 25, for sample (h) was $[\alpha]^{23}\text{D}$ +16.5° and on sample (i) $[\alpha]^{23}\text{D}$ +15.3°. The ash content of the final preparations was less than 0.01%. Carbon appeared in the crucible occasionally but was negligible in amount.

***d*-Tyrosine.**—One commercial material and a student preparation from silk were used in making five different combustion samples.

(a) A sample from Hoffmann–La Roche was used without additional purification.

(b) A portion of (a) was once recrystallized from hot water.

---

[5] Steiger, *J. Biol. Chem.*, **86**, 695 (1930).

[6] Fischer and Warburg, *Ber.*, **38**, 3997 (1906).

(c) The student preparation was once recrystallized from water.

(d) A portion of (a) was twice recrystallized from 275 parts of water.

(e) The student preparation was treated with norite and recrystallized three times from 250 parts of water.

The nitrogen content was found to be the theoretical. The rotation was determined on a solution containing 0.863 g. of tyrosine in 25 cc. of 4% hydrochloric acid and gave $[\alpha]^{24}\text{D}$ −10.8. The ash content of all of the preparations except (*a*) was entirely negligible. With one exception there was no evidence of the production of carbon

TABLE I

THE EXPERIMENTAL DATA AT 25°

Sample	P_2O_5 vac.	Desiccation vac. 70° hours	Oven 100°	True mass	Total heat evolved cal.	Cal. from HNO_3	Cal. from oil	Cal. from paper and EIt	$-\Delta U_B/m$, cal. g^{-1}	Deviation
dl-Alanine (e)			72	1.24330	6326.1	18.8	829.6	17.2	4341.3	1.4
dl-Alanine (e)			216	1.18485	6327.3	17.8	1151.1	16.4	4339.7	−0.2
dl-Alanine (g)			264	1.31045	6357.5	20.0	634.2	16.9	4339.3	− .6
dl-Alanine (h)			168	1.22810	6664.9	18.2	1299.4	16.8	4340.4	.5
dl-Alanine (l)			192	1.28310	6445.5	19.4	840.5	16.4	4340.4	5
dl-Alanine (p)			114	1.26470	6339.8	18.9	815.9	18.0	4338.5	−1.4
dl-Alanine (m)			192	1.34295	6413.2	18.4	548.8	18.1	4339.6	−0.3
Mean									4339.9	± .7
d-Arginine (a)	192			1.22515	6326.2	24.2		17.1	5129.9	− .2
d-Arginine (a)	280	90		1.22880	6345.2	25.4		16.5	5129.6	− .5
d-Arginine (a)	280	90		1.22025	6302.6	25.6		17.1	5130.1	.0
d-Arginine (b)	170			1.22860	6346.2	25.6		17.8	5130.1	.0
d-Arginine (e)	240	143		1.10065	6342.6	25.8	651.8	17.6	5131.0	.9
d-Arginine (e)	2400	143	67	1.09845	5346.0	25.3	666.6	17.9	5131.1	1.0
d-Arginine (g)	2400		87	1.19055	6335.1	23.8	186.2	17.4	5130.2	+0.1
d-Arginine (g)	21		40	1.16700	6325.0	24.4	297.4	17.3	5129.3	− .8
d-Arginine (g)	24	116	45	1.21625	6343.7	25.3	61.6	17.9	5129.6	− .5
Mean									5130.1	± .5
Glycine (a)	912		72	2.02560	6319.1	17.8		16.9	3102.5	− .5
Glycine (c)	912		72	2.01465	6286.8	17.1		17.3	3103.5	.5
Glycine (d)	336		96	2.02080	6305.7	17.0		17.9	3103.1	+ .1
Glycine (e)	240		120	2.02715	6325.5	18.5		16.9	3102.9	− .1
Mean									3103.0	± .3
dl-Leucine (a)	240			0.97280	6367.9	12.6		17.4	6515.2	.0
dl-Leucine (a)	1032			1.03670	6784.3	13.8		16.8	6514.6	− .6
dl-Leucine (a)	1176			0.97110	6356.6	12.8		17.8	6514.4	− .8
dl-Leucine (a)			144	.96940	6347.5	13.3		17.6	6515.9	.7
dl-Leucine (a)			216	.96870	6343.7	14.3		17.5	6515.7	.5
dl-Leucine (b)	192			.07060	6353.8	13.0		17.2	6515.3	.1
Mean									6515.2	± .5
d-Leucine (a)	504			.96535	6326.5	14.1		17.0	6521.6	.7
d-Leucine (a)	1176			.96685	6335.8	13.6		16.6	6521.7	.8
d-Leucine (b)	538			.97125	6363.0	14.3		17.0	6519.5	−1.4
Mean									6520.9	±1.0
l-Leucine (f)	192			.97070	6360.8	14.7		16.9	6520.2	−0.5
l-Leucine (g)	144			.96965	6353.9	14.2		15.8	6521.7	1.0
l-Leucine (h)	1032			.97085	6362.7	14.7		17.8	6520.6	−0.1
l-Leucine (h)	96		24	.97160	6366.0	13.4		16.2	6521.7	1.0
l-Leucine (j)	120			.96990	6354.0	12.5		17.0	6520.8	0.1
l-Leucine (j)			38	.97200	6366.9	12.8		17.3	6519.4	−1.3
Mean									6520.7	±0.7
l-Tyrosine (a)	264			1.06525	6253.8	10.4		17.3	5844.6	1.7
l-Tyrosine (a)			200	1.08105	6345.0	11.2		17.4	5842.8	0.1
l-Tyrosine (a)			240	1.08090	6344.5	10.7		17.1	5843.9	1.0
l-Tyrosine (c)	384			1.07945	6333.8	10.8		17.0	5841.7	−1.2
l-Tyrosine (c)	144		72	1.07780	6324.5	10.8		16.7	5842.6	−0.3
l-Tyrosine (d)	120		264	1.08070	6343.3	11.8		17.4	5842.7	− .2
l-Tyrosine (d)	128		264	1.07190	6294.2	12.5		18.2	5843.4	.5
l-Tyrosine (d)			264	0.97780	6330.5	11.3	588.2	17.7	5842.9	.0
l-Tyrosine (e)			216	.96980	6337.1	10.5	644.5	17.1	5841.4	−1.5
Mean									5842.9	±0.7

TABLE II

SUMMARY OF DERIVED DATA AT 25°

Substance	Formula	Mol. wt.	Density	$-\Delta U_B$, kcal. mole^{-1}	$-\Delta U_R$, kcal mol	$-\Delta H_R$, kcal. mole^{-1}	$-\Delta H°_f$, kcal. mole^{-1}	$\Delta U_R/dT$ mole^{-1} cal.
dl-Alanine	$C_3H_7O_2N$	89.093	1.37	386.65 ± 0.12	386.40 ± 0.14	386.55 ± 0.14	135.26	38
d-Arginine	$C_6H_{14}O_2N_4$	174.201	1.1	893.67 ± .25	893.18 ± .30	893.48 ± .30	150.16	78
Glycine	$C_2H_5O_2N$	75.067	1.601	232.93 ± .08	232.72 ± .10	232.57 ± .10	126.69	18
d-Leucine	$C_6H_{15}O_2N$	131.169	1.29	855.34 ± .18	854.97 ± .21	856.01 ± .21	153.47	72
l-Leucine	$C_6H_{13}O_2N$	131.169	1.29	855.31 ± .17	854.95 ± .20	855.98 ± .20	153.49	72
dl-Leucine	$C_6H_{13}O_2N$	131.169	1.29	854.59 ± .18	854.19 ± .21	855.23 ± .21	154.25	72
l-Tyrosine	$C_9H_{11}O_3N$	181.184	1.456	1058.64 ± .30	1057.90 ± .37	1058.34 ± .37	165.54	60

and in this case the correction was less than 0.01%. We were unable to obtain combustion results on preparation (b) due to loss by spattering.

The experimental results of the combustions on these seven compounds are given in Table I.

In addition to the experimental values given in Table I we have also calculated the quantities $-\Delta U_B$, $-\Delta U_R$, $-\Delta H_R$ and $-\Delta H_f$ which we have defined previously.[1b] We have also calculated the temperature coefficient of ΔU_R. In the calculation of $-\Delta H°_f$ we have used for the heats of formation of carbon dioxide[7] and water[8] the values −94,240 and −68,313 cal., respectively, for their heats of formation. Due to the uncertainty in Roth's value for the heat of formation of carbon dioxide and to the fact that data now being obtained at the National Bureau of Standards,[8a] and at the Carnegie Institute of Technology indicate that the present value probably is too high, we have not corrected this datum for the change in atomic weight of carbon. Because of the above uncertainty, we have not set any limits of error for the values of $-\Delta H°_f$.

Discussion of the Results

As in the previous papers of this series we have chosen as the limit of error the extreme deviation in each case and have allowed for an additional uncertainty of 10% in the Washburn correction.

Some of these compounds have been burned by earlier workers. Wrede[9] has burned *dl*-alanine and glycine, Emery and Benedict[10] have burned *l*-tyrosine, Fischer and Wrede[11] have burned leucine, Stohmann and Langbein[12] have burned glycine and leucine, and Berthelot and Andre[13] have burned leucine and *l*-tyrosine. With the exception of Wrede none of the above authors have included enough data for correction in terms of the modern thermochemical standards. It further may be remarked that the precision of the measurements of Stohmann and Langbein and of Berthelot and Andre is so low that little could be gained from such a correction, their results are hence only of historical interest. We have, however, made approximate corrections and have compared some of these early data with ours in Table III. In the case of *l*-tyrosine the corrected value of Emery and Benedict[10] agrees exactly with ours. This does not necessarily have any significance. The value of *l*-tyrosine as reported by Emery and Benedict[10] was originally based on the percentage of carbon dioxide as determined by analyses. In applying our corrections we have arbitrarily reduced their value back to that of the mass of the sample used. Their carbon dioxide analyses correspond to only 99.0% of the theoretical value and they state that the mass of the material was unchanged by prolonged desiccation. Hence either their carbon dioxide analyses were very poor or the sample used was very impure.

TABLE III

COMPARISON OF THE PRESENT DATA AT 25° WITH VALUES FROM THE LITERATURE

Substance	Reference	Older data, kcal. mole^{-1}	New value kcal. mole^{-1}	Difference %
dl-Alanine	9	387.00 ± 0.19	386.55 ± 0.14	0.12
Glycine	9	233.04 ± .25	232.57 ± .10	.20
Glycine	12	234.00 ± .70	232.57 ± .10	.62
l-Leucine	12	854.6 ± 1.30	855.98 ± .20	−.16
l-Tyrosine	13	1071.0 ± 2.3	1058.34 ± .37	1.20
l-Tyrosine	10	1058.34 ± 2.4	1058.34 ± .37	0.00

In the case of leucine we are able to compare the heats of combustion of the two optically active forms and find that they are identical, which is to be expected from the theory of optical isomerism. We are also able to compare the heats of com-

---

(7) Parks and Huffman, "The Free Energies of Some Organic Compounds," The Chemical Catalog Co., New York, 1932.

(8) Rossini, *B. Standards J. Research*, **6**, 34 (1931).

(8a) Private communication from Dr. F. D. Rossini.

(9) Wrede, *Z. physik. Chem.*, **75**, 91 (1910).

(10) Emery and Benedict, *Am. J. Physiol.*, **28**, 301 (1911).

(11) Fischer and Wrede, *Sitzber. kg. preuss. Akad. Wiss.*, 687 (1904).

(12) Stohmann and Langbein, *J. prakt. Chem.*, **44**, 336 (1891).

(13) Berthelot and Andre, *Ann. chim. phys.*, [6] **22**, 5 (1891).

bustion of the active and inactive forms of alanine and leucine and in both cases we find that the active form has the higher value and in these two cases values which are the same within the experimental error, namely, 0.68 and 0.77 kcal., respectively. This result may also have been expected, as it is well known that the properties of the inactive form are often markedly different from those of the active form.

Summary

1. The experimental values of the isothermal heats of combustion at constant volume and at 25° of seven amino acids are given.

2. The methods of preparation and purification and photomicrographs of the crystalline condition of these amino acids are given.

3. These new values for the heats of combustion have been compared with older values when available.

4. In two cases the optically active form has been found to have a higher heat of combustion than the inactive form.

PASADENA, CALIF. RECEIVED AUGUST 13, 1937

[CONTRIBUTION FROM THE WILLIAM G. KERCKHOFF LABORATORIES OF THE BIOLOGICAL SCIENCES OF THE CALIFORNIA INSTITUTE OF TECHNOLOGY]

Thermal Data. VIII. The Heat Capacities, Entropies and Free Energies of Some Amino Acids

BY HUGH M. HUFFMAN AND EMORY L. ELLIS

In the preceding paper[1] of this series we have presented accurate combustion data on seven amino acids. In this paper we present the low temperature heat capacity data of four amino acids from the above-mentioned group. These data have been used to calculate the entropies of the compounds, and the entropy data in conjunction with the corresponding heats of formation to calculate the free energies of the amino acids. In one case we have determined experimentally that the entropies of the active and inactive forms are the same. We have made further use of this observation in calculating the free energies of two substances whose heat capacities were not measured.

Experimental

In principal the method of Nernst was employed with an aneroid calorimeter to determine the "true" specific heats. The details of the method have been described elsewhere[2] so that only a brief account will be given.

In brief it consists in supplying a measured amount of heat to a gold calorimeter containing the substance under investigation. To ensure rapid thermal equilibrium the substance is pressed into dense pellets, about 2 mm. thick, and spaced along the centrally located thermocouple well by means of thin gold disks which are in good thermal contact with the walls of the calorimeter. The electrical measurements of current and voltage are made on a "White" double potentiometer by the proper use of accurately calibrated resistances. Time measurements are made by means of a calibrated stop watch. The temperature measurements are made by means of the White potentiometer in conjunction with a single-junction copper-constantan thermocouple. This couple is periodically standardized against one of the couples calibrated in the investigation of Giauque, Johnston and Kelley.[3]

Materials.—The *dl*-alanine was a commercial product obtained from Pfanstiehl, and was subjected to several fractional crystallizations from water. The nitrogen determined by the Kjeldahl method was the theoretical. The ash content was of the order of 0.03 to 0.04%. This material crystallized in the form of long narrow rods.

The *dl*-leucine used in this investigation was prepared in the Carlsberg laboratories and was carefully purified in this Laboratory by K. Linderstrøm-Lang. The nitrogen content was the theoretical and the ash content negligible.

The *d*-arginine was loaned to us for this investigation by Hoffmann-LaRoche. It was fractionally crystallized several times from water taking care to exclude carbon dioxide. The nitrogen as determined by microkjeldahls was low, only about 97% of the theoretical value being found. The ash content was negligible.

l-Tyrosine was prepared from silk by the method of Abderhalden and Teruuchi.[4] It was purified carefully by crystallizing several times from water. The nitrogen content was the theoretical and the ash content negligible.

In view of the accuracy of the measurements and the purity of the compounds involved the error in the experimental results is probably less than 1%.

The specific heat data in terms of the defined conventional calorie and for true mass appear in Table I.

Discussion

Entropies of the Compounds.—From the data in Table I we have calculated the entropies of the compounds in the usual manner using the extra-

---

[1] Huffman, Ellis and Fox, THIS JOURNAL, **59**, 2144 (1937).

[2] Parks, *ibid.*, **47**, 338 (1925).

[3] Giauque, Johnston and Kelley, *ibid.*, **49**, 2367 (1927).

[4] Abderhalden and Teruuchi, *Z. physiol. Chem.*, **48**, 528 (1906).