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## Thermal Data. IX. The Heats of Combustion of Hippuric and Succinic Acids and a Proposal for the Use of Hippuric Acid as a Secondary Standard in Combustion Calorimetry

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The use of benzoic acid as the standard material for determining the energy equivalent of the calorimetric system in bomb calorimetry has been accepted by the Commission Permanente de Thermochimie de la Union Internationale de Chimie.<sup>1</sup> The question of the desirability of secondary standards of equal reliability has been discussed at length by Verkade and Coops<sup>2</sup> and the principle was adopted by the above-mentioned commission in 1933. At a later meeting of this commission (1936)<sup>3</sup> succinic acid was formally adopted as a secondary standard and a preliminary value was given for its isothermal heat of combustion. We have used this material to check our calorimetry and in view of the fact that a final value has not been accepted, we have thought it desirable to put our results on record.

Our work in the field of combustion calorimetry has been principally with compounds containing nitrogen, and it has occurred to us that it would be highly desirable to have a nitrogen-containing substance as a secondary standard. Then the conditions holding in the combustion of the nitrogen-containing standard substance would approach more nearly the conditions holding in the combustion of nitrogen compounds, and the burden of establishing the validity of the correction for the formation of nitric acid will rest primarily upon the investigators who are establishing values for the heat of combustion of the nitrogen-containing standard substance in terms of electrical energy.

Among the various nitrogen containing substances which we have burned, we were particularly attracted by certain properties of hippuric acid which led us to believe that it might make an excellent standard material. We decided to investigate these properties more thoroughly with the intent of suggesting it as a secondary standard. Since concluding our work we have found that this

material was used by Stohmann<sup>4</sup> for the same purpose and according to Verkade and Coops<sup>2</sup> it is still used as a standard material in technical work.

### Calorimetric Method

The method and apparatus have been described in previous communications.<sup>5</sup> Two minor changes have been made in our system: (1) we have introduced a new resistance thermometer; and (2) we have equipped the calorimeter with a new heating coil enclosed in a gold-plated copper sheath. Due to these changes the energy equivalent of the calorimeter is different but the precision of our measurements is unchanged. In a series of nine calibration runs made during the course of this investigation, using Bureau of Standards benzoic acid no. 39e, we have obtained as the energy equivalent of our calorimeter the value 3153.2 cal. with an extreme deviation of 0.3 and a mean deviation of  $\pm 0.2$  cal.

All of our combustions have been made in a Parr bomb having a volume of 0.380 liter, with an initial oxygen pressure of 30 atm. and with 1 ml. of water added to the bomb. We have used the constant range method exclusively and all of the combustions of benzoic acid and the other materials have started at 24.00° and, with rare exceptions, ended at 26.00  $\pm$  0.01°. All of the ignitions were effected by the platinum wire technique<sup>5a</sup> in which the joule input is maintained very constant by carefully controlling the e. m. f., the resistance in the line and the time, the latter by means of a time switch operated by a synchronous motor.

### Units and Corrections

The unit of energy used throughout this investigation is the defined conventional calorie which is derived from the international joule by multiplying by the factor 1.0004/4.185. It is to be noted that we are using for the isothermal heat of combustion of benzoic acid at 25.0° the value 26,419 international joules per gram true mass as determined by Jessup and Green.<sup>6</sup> This becomes 6315.3 cal. when multiplied by the factor given above. It is to be noted further that this value holds only when the combustions are carried out under the conditions specified by Washburn,<sup>7</sup> namely, at 25.0° with a total initial oxygen pressure of 30 atm. and when the ratios of the initial weight of water and of benzoic acid to the volume of the bomb in liters are both equal to 3. When the conditions of the combustion deviate from those given above, a different value must be used which can be calculated from the equa-

(1) Premier Rapport de la Commission Permanente de Thermochimie de la Union Internationale de Chimie, Paris, 1934.

(2) Verkade and Coops, *Rec. trav. chim.*, **43**, 561 (1924).

(3) *Revue Analytique and Critique de Thermochimie Organique, Appendices au 1<sup>er</sup> Rapport de la Commission Permanente de Thermochimie, Paris, 1936.*

(4) Stohmann and Langbein, *J. prakt. Chem.*, [2] **49**, 99 (1894).

(5) (a) Huffman and Ellis, *THIS JOURNAL*, **57**, 41 (1935); (b) Stiehler and Huffman, *ibid.*, **57**, 1734 (1935).

(6) Jessup and Green, *Bur. Standards J. Research*, **13**, 496 (1934).

(7) Washburn, *ibid.*, **10**, 525 (1933).

TABLE I  
THE ISOTHERMAL HEAT OF COMBUSTION OF SUCCINIC ACID AT 25.0°

Sample	Desiccation hours in		True mass, g.	Total heat evolved, cal.	Cal. from HNO <sub>3</sub>	Cal. from paper & Et	$-\Delta U_B/m$ cal. g. <sup>-1</sup>	Dev.
	Vacuum 50-55°	Oven 105-110°						
a	45		2.08292	6310.5	0.1	16.8	(3021.5) <sup>a</sup>	(-1.6)
a		45	2.09153	6340.6	.1	18.1	3022.8	-0.3
a	45	16	2.07861	6301.4	.1	17.8	3022.9	-.1
b		18	2.09052	6337.7	.1	17.8	3023.1	.0
b		18	2.09107	6339.1	.1	17.2	3023.2	.1
b		18	2.09254	6345.5	.2	17.7	3023.9	.8
b		18	2.09103	6340.1	.2	18.1	3023.4	.3
b		18	2.09152	6339.8	.8	17.8	3022.7	-.4
b		68	2.09332	6346.5	.8	18.2	3023.1	.0
						Mean	3023.1	± .3

<sup>a</sup> Sample probably not completely dried; not used in obtaining mean.

tion given by Jessup and Green.<sup>6</sup> Since our conditions are slightly different from the standard ones, we have calculated that we should use the value 6315.1 cal. In our earlier communications we have used the value 6315.3; however, since we are going to compare our data on succinic acid with those obtained under conditions considerably different, we have thought it desirable to apply the same sort of corrections in each case.

The European workers use a different value for the heat of combustion of benzoic acid, namely, 6319.0 cal./gram *in vacuo* at 20.0° and under the standard conditions. This becomes 6317.9 cal. at 25.0°, hence, their results must be multiplied by the factor 0.99959 to convert to our calorie. Furthermore, since all of their results are for a temperature of 20.0° or lower, they must be converted to 25.0° for comparison with our data. We have calculated that the temperature coefficient of  $-\Delta U_B/m$  for succinic acid is -0.24 cal./gram/degree.

A correction is always necessary for nitric acid formed due either to the nitrogen in the compound or to nitrogen present in the oxygen. With CHO compounds we have found that this correction is usually less than 1 cal. Consequently the correction need not be known with a particularly high percentage accuracy. With compounds containing nitrogen we have found that this correction may become as high as 0.6% of the total heat given to the calorimeter. In this case it becomes necessary that this correction should be known with an accuracy of 1% or better. In correcting for the nitric acid, in former publications, we have used the value 14,800 cal. as the heat evolved when 1 mole of nitric acid is formed from gaseous nitrogen, oxygen and liquid water at 25° and at constant volume. Beckers and Roth<sup>8</sup> in a more recent and direct determination give the value for the above process at 20° of 13,820 cal., this corrected to 25.00° becomes 13,960 cal. and is the value we have used in this publication. In determining the amount of nitric acid formed, we have titrated the bomb washings with dilute aqueous sodium hydroxide using brom cresol green as the indicator. As a further check on the validity of this correction we have, in several cases, made a direct determination of the nitrogen in the bomb washings by reducing with Arndts metal and determining as ammonia. The agreement between the two methods of analysis was of the order of 1%. In addition

(8) Beckers and Roth, *Z. Elektrochem.*, **40**, 835 (1934).

we have examined the bomb washings for nitrous acid, by an extremely sensitive colorimetric test, and have found only the merest traces.

All of our values of  $-\Delta U_B/m$  are given for the isothermal process. In correcting to this condition we have used the method of Washburn.<sup>7</sup> Since our values are calculated for a temperature which is the mean of our initial and final temperatures, it is necessary to know the heat capacity of the substance burned, in addition to the heat capacity of the products of the reaction. This method is particularly useful as the temperature coefficient of the reaction may be directly calculated from these data.

#### Preparation, Purification and Heats of Combustion of the Compounds

**Succinic Acid.**—(a) A product obtained from Eastman had been crystallized repeatedly from water by W. R. Bergren of this Laboratory for use in another investigation. (b) Eastman succinic acid was subjected to four fractional crystallizations from water especially for this investigation.

Titration of this material with dilute sodium hydroxide gave the theoretical results. The ash content as determined from the residues left in the crucible after combustion was completely negligible. The results of the combustions on these preparations are given in Table I.

**Hippuric Acid.**—We have obtained hippuric acid from three commercial sources for this investigation, namely, the Amino Acid Manufacturers, Eastman and Pfanstiehl. The first named source supplies this material in two qualities, A. P. (analytically pure) and C. P. From these original materials we have made eight preparations for combustion purposes.

(a) The A. P. material from the Amino Acid Manufacturers was subjected to one recrystallization from water.

(b) The C. P. material from the Amino Acid Manufacturers was used without further purification.

(c) Eastman hippuric acid was once recrystallized from water.

(d) The material from Pfanstiehl was used without further purification.

(e) A portion of sample (d) was once recrystallized from water.

(f) A portion of the A. P. acid from the Amino Acid Manufacturers was subjected to six fractional crystallizations from water.

(g) The solute remaining in the first two mother liquors from preparation (f) was crystallized three times from absolute alcohol.

(h) The solute remaining in the last three mother liquors from preparation (f) were crystallized three times from absolute alcohol.

(i) Part of sample (h) was dissolved in water and the solvent evaporated off.

Titration of the A. P. quality hippuric acid with dilute

sodium hydroxide gave theoretical results. The ash content of all the preparations was either undetectable or completely negligible. The purity of the compounds is further indicated by the complete agreement of the combustion results obtained with samples (a) and (f) which were from the same original preparation with one and six recrystallizations, respectively. In the one case where combustion data indicated that the compound contained a small amount of impurity, sample (d), one recrystallization,

TABLE II  
THE ISOTHERMAL HEAT OF COMBUSTION OF HIPPURIC ACID AT 25.0°

Sample	Desiccation hours in P <sub>2</sub> O <sub>5</sub> vac.	Oven 105-110°	True mass, g.	Total heat, evolved, cal.	Cal. from HNO <sub>3</sub>	Cal. from paper and Elt	- $\Delta U_B/m$ cal. g. <sup>-1</sup>	Dev. from group mean	Dev. from general mean
a	110		1.11890	6325.1	10.0	16.4	5629.3	0.3	0.7
a	120		1.12399	6354.1	10.4	17.4	5628.4	-.6	-.2
a	288		1.12208	6344.2	10.4	16.7	5629.7	.7	1.1
a	72 <sup>a</sup>		1.11811	6320.9	9.8	17.4	5628.8	-.2	0.2
a		64	1.12226	6345.2	10.1	18.5	5628.4	-.6	-.2
a		306	1.12105	6338.6	10.4	17.8	5629.0	.0	.4
a	96	18 <sup>b</sup>	1.12044	6334.3	10.0	16.6	5629.6	.6	1.0
a	96	41 <sup>b</sup>	1.11646	6311.6	9.9	17.3	5628.8	-.2	0.2
							5629.0	± .4	
b	120		1.11814	6319.4	10.4	16.6	5627.5	-1.0	-1.1
b		120	1.12299	6348.5	10.3	17.0	5628.9	0.4	0.3
b		217	1.12090	6336.3	10.0	17.2	5628.6	.1	.0
b		419	1.11924	6326.7	9.9	16.6	5629.0	.5	.4
							5628.5	± .5	
c		48	1.11968	6328.9	10.4	17.2	5627.7	-.3	-.9
c		64	1.11779	6322.8	10.3	20.8	5628.7	.7	.1
c		92	1.11957	6327.6	10.3	17.0	5627.4	.6	-1.2
c		92	1.12053	6334.0	10.1	17.4	5628.1	.1	0.5
							5628.0	± .4	
d		246	1.12066	6334.0	10.1	17.8	5627.1	.6	(-1.5)
d		246	1.12080	6333.7	10.4	17.6	5626.0	-.5	(-2.6)
d		246	1.12183	6339.4	10.3	17.2	5626.4	-.1	(2.2)
							5626.5	± .4	
e		69	1.11920	6327.2	10.0	17.4	5628.8	.5	0.2
e		69	1.12024	6331.2	9.8	16.7	5628.0	-.3	-.6
e		69	1.12024	6331.7	10.0	16.8	5628.1	-.2	-.5
							5628.3	± .3	
f	117		1.12072	6335.5	9.9	17.6	5628.5	.0	-.1
f	162		1.12353	6351.8	10.0	17.8	5628.7	.2	.1
f	101	39	1.11809	6321.7	10.0	17.5	5629.4	.9	.8
f	101	39	1.12105	6335.9	10.1	16.5	5628.0	-.5	-.6
f	101	39	1.12055	6332.7	10.0	16.1	5628.1	-.4	.5
							5628.5	± .4	
g	25	70	1.12837	6388.6	10.2	17.4	5637.4	+ .1	(8.8)
g		130	1.11904	6334.3	10.1	16.7	5636.5	-.8	(7.9)
h	22	219	1.11990	6341.7	10.0	17.4	5638.1	+ .8	(8.5)
							5637.3	± .6	
i	80	24	1.12111	6338.4	10.1	17.5	5628.8		0.2
			Mean of group means (omitting d, g and h)				5628.5	± .4	
			Mean of all (omitting d, g and h)				5628.6	± .5	

<sup>a</sup> Plus 68 hours *invacuo* at 80-85°. <sup>b</sup> Oven temp. 140-145°

sample (e), served to bring the combustion results into excellent agreement with those on the other preparations. From these data we feel that we are justified in making the statement that hippuric acid may be obtained readily in the high state of reproducible purity necessary for a combustion standard.

Three combustions were made on samples (g) and (h) which had been crystallized from alcohol. These combustions gave a mean result which was 0.154% higher than the best value obtained on the samples crystallized from water. There are four possible explanations for this difference: (1) failure to remove all of the solvent, alcohol, which would lead to a high result, (2) failure to remove all the water from the other preparations, (3) the preparations were of different purity, and (4) polymorphism. We are not prepared to state positively at this time to which of these possible causes the high result is due. We are investigating this phenomenon further and hope to be able to make a report in a future communication. The results of our combustions on hippuric acid are given in Table II.

**Properties of Hippuric Acid.**—Before a substance may be considered desirable as a standard, it should fulfil certain minimum requirements. We shall put down these requirements, and our findings under each, in the following section.

(1) *It should be readily obtainable in a pure state.* We have already shown in the preceding section that this material may be purchased from a number of commercial sources in what is apparently a very high state of purity. In addition we have shown that it is purified easily by crystallization from water. We might add that the cost of this material is nominal, in view of its high quality.

(2) *It should be easy to dry and should remain so without special precautions.* We have performed two sorts of overlapping experiments to check the drying of this material. First, we have done combustions on samples which had been dried under different conditions and for varying lengths of time under any one condition. Second, we have attempted to ascertain when the samples were dry by following the loss in weight with time under certain fixed conditions. We had hoped at the start of this investigation that this material would have an inappreciable vapor pressure and could be dried to a constant weight. This did not prove to be true, but we did find that when all of the variables except the time were fixed within certain limits, the samples seemed to approach rapidly a steady state where the rate of loss in weight became nearly constant. This constancy of rate was attained in twenty-four to forty-eight hours depending upon the method of drying and upon the residual water in the sample.

Duplicate drying experiments were carried out on the crystalline material, the material ground in an agate mortar and on the pelleted material. The drying was done under the following conditions, *in vacuo* over phosphorus pentoxide at room temperature which was in the neighborhood of 24°, *in vacuo* over drierite at 80–85°, in air at 105–110° and in air at 140–145°. The results of the experiments in which the change in mass was followed are summarized in Table III. All of the samples were dried and weighed in platinum crucibles. Most of these samples were burned at the conclusion of the drying experiment. Reference to Table II shows absolutely no trend of the combustion values with

the time of drying, under any of the conditions, so we believe there is no question as to the complete removal of sorbed water from these samples. From the data of Table III we might conclude that the simplest method to use is to pellet the material and place *in vacuo* over phosphorus pentoxide for several days. In fact we would recommend that the stock of this material either crystalline or pelleted be stored in this manner until ready for use. The loss in mass under these conditions is so small that pellets could be made, adjusted to weight and stored for long periods of time without appreciable changes in mass. This is distinctly not true of benzoic acid.

TABLE III

## DRYING OF HIPPURIC ACID

Expt.	Initial wt. of samples about 1.12 grams		Decrease in mass.	
	Total	Increment	No. 1	No. 2
Expt. A, Sample (e), pellets in P <sub>2</sub> O <sub>5</sub> vac. at room temp.				
	0	0		
	23	23	1.01	0.77
	51	28	0.01	.01
	117	66	.01	.03
	162	45		.01
Expt. B, Sample (a), pellets <i>in vacuo</i> over drierite, 80–85°				
	0	0		
	22	22	0.32	0.24
	68	46	.34	.37
Expt. C, Sample (b), crystals, air, 105–110°				
	0	0		
	16	16	4.43	2.90
	39	23	0.98	1.85
	60	21	.12	0.09
	105	45	.13	.15
	173	68	.20	.36
	195	22	.04	.03
	264	69	.09	.13
	404	140	.30	.55
Expt. D, Sample (b), crystals powdered, air at 105–110°				
	0	0		
	21	21	0.88	*
	42	21	.07	0.06
	59	17	.01	.03
	78	19	.06	.04
Expt. E, Sample (b), material from Expt. D pelleted, air at 105–110°				
	0	0		
	16	16	1.00	0.17
	34	18	0.03	.02
	54	20	.00	.01
	123	69	.06	.11
Expt. F, Sample (a), pellets, air at 140–145° <sup>b</sup>				
	0	0		
	18	18	1.60	
	41	23		1.08

\* A portion of this sample was spilled after this weighing. <sup>b</sup> Two pellets in one crucible. After the first heating period one was removed and burned.

We also have performed experiments to determine the gain in mass of this material when exposed to the air of the room and also in a closed space saturated with water vapor. The temperature of the room during these experiments was about 24° and the relative humidity between 60 and 70. The results are summarized in Table IV. From the data it may be seen that the increase in mass is very small, less than 0.01% after twenty-one days for pelleted material. We have noted many times, though not always, that the pellets begin to increase in mass immediately upon removal from the desiccator, thus one may observe an increase in mass of 0.01 to 0.04 mg. during the course of the weighing. This in our particular case is just about the order of accuracy of our weighings and is of no consequence. We have also noticed the same phenomenon with succinic acid.

TABLE IV

## HYGROSCOPICITY OF HIPPURIC ACID

Expt.	Time of exposure, days		Increase in mass, increment in mg.	
	Total	Increment	No. 1	No. 2
Initial wt. of samples about 1.12 grams				
Time of exposure, days				
Increase in mass, increment in mg.				
No. 1				
No. 2				
Expt. A, Sample (b), pellets, exposed to air of room, previously dried at 105–110°, 7–8 days				
	0	0		
	1	1	0.06	0.05
	2	1	.00	.00
	11	9	.03	.03
	21	10	.02	.02
	Total increase		.11	.11
Expt. B, <sup>b</sup> Sample (b), ground, exposed to air of room and saturated water vapor. Previously dried at 105–110° for 78 hours				
	0	0		
	21	21 <sup>a</sup>	0.07	0.07
	69	48	-.03	-.01
	90	21	.02	.00
	Total increase		.06	.06

<sup>a</sup> In saturated water vapor. <sup>b</sup> Time in hours.

(3) *It should be completely stable at elevated temperatures.* This qualification is included so that the substance may be dried completely by treatment at elevated temperatures. As we have shown in the preceding section, such a procedure is not necessary with hippuric acid. However, we have demonstrated the complete stability of this compound up to temperatures as high as 140°. We have made combustions on samples which have been heated at 105–110° for periods ranging from thirty-nine to four hundred nineteen hours, and on samples heated at 140–145° for eighteen and forty-one hours. By reference to Table II, all of these combustions are seen to be in agreement with the other members of their group and show absolutely no evidence of a trend with heating time.

(4) *It should not be volatile.* The data in Table III show that the vapor pressure of this material must be extremely low. From the data of experiment A, taking the worst case, a pellet exposed in phosphorus pentoxide vacuum decreased in mass less than 0.005% in one hundred thirty-nine hours. Taking the data from experiment B, in which the conditions were probably most favorable for

vaporization, since the oven was being evacuated continuously, the loss in mass was only about 0.03% in a period of forty-six hours. From these data it may be concluded that there is not the slightest danger of significant error from this source.

(5) *It should pellet readily.* The material either crystalline or powdered is made into pellets easily. These are somewhat more fragile than those made from benzoic or succinic acid. This need not lead to trouble if the pellets are weighed directly in the crucible in which they are to be burned.

(6) *It should be readily inflammable.* Hippuric acid is ignited readily by the platinum wire technique,<sup>5a</sup> which is now the standard method used in this Laboratory. The filter paper is adjusted to such a weight as will supply 15 to 16 cal. Under these conditions and out of some thirty-odd combustions we have never had a failure to fire.

(7) *It must burn completely.* We have observed in approximately one-quarter of our combustions small residues of carbon in the crucible. Upon weighing these residues we have found them to be of the order of 0.01 to 0.02 mg. or the equivalent of about 0.1 to 0.2 cal., an entirely negligible quantity. We have observed the same in burning benzoic or succinic acid but again in such small amounts as to be entirely negligible. We have tested the products of combustion for carbon monoxide in two cases, using the technique of Eiseman, Weaver and Smith<sup>9</sup> with entirely negative results. In check experiments we found that this method was easily capable of detecting as little as 0.1 to 0.2 cc. of carbon monoxide in a volume of gas equivalent to that present in a combustion experiment. In none of our combustions have we found the slightest evidence of spattering.

## Discussion of the Results

Succinic acid has been studied carefully in three other investigations by Beckers,<sup>10</sup> Keffler<sup>11</sup> and Beckers and Roth<sup>12</sup> so it is of considerable interest to compare our data with those of these workers. The results of all of these investigators have been obtained under conditions which are different from those of this investigation and in addition they have used a different calorie. For these reasons certain corrections must be applied to the data before they are strictly comparable. These corrections are as follows. (1) All of these workers have calibrated their calorimeters with benzoic acid but none have burned this substance under exactly standard conditions, hence, a small correction must be applied for this deviation. (2) Becker's<sup>10</sup> results have been determined for the temperature 18.8° and those of Keffler<sup>11</sup> and of Roth and Beckers<sup>12</sup> at 20° and the results of this investigation are for 25.0°; hence, a correction must be ap-

(9) Eiseman, Weaver and Smith, *Bur. Standards J. Research*, **8**, 669 (1932).

(10) Beckers, *Bull. soc. chim. Belg.*, **40**, 571 (1931).

(11) Keffler, *J. Phys. Chem.*, **38**, 717 (1934).

(12) Beckers and Roth, *Z. physik. Chem.*, **A179**, 450 (1937).

plied to bring them all to the same temperature. (3) Since the bomb conditions of the various investigators were not the same, the Washburn correction would be different in each case; hence, to be comparable the results must be corrected to standard bomb conditions. (4) Finally the data must all be expressed in the same calorie.

We have applied these corrections and the corrected data are given in Table V in terms of our calorie and at 25.00°. The values of  $-\Delta U_R/m$  have been obtained by the method of Washburn<sup>7</sup> and the values of  $-\Delta U_B/m$  have been corrected to a standard bomb condition which we have chosen to be the same as that given by Washburn<sup>7</sup> for benzoic acid except that  $m/v = 3R$  in which  $R$  is the ratio of the heat of combustion of benzoic acid to that of succinic acid. The data in each column are then comparable. These data may be converted to the reaction at 20.0° and to the calorie used by the European workers by increasing the values in the table by 2.4 cal.

TABLE V

COMPARISON OF THE DATA ON SUCCINIC ACID AT 25.0° AND FOR STANDARD CONDITIONS

Temp.	coeff. of $-\Delta U_B/m = -0.24 \text{ cal. g.}^{-1} \text{ degree}^{-1}$			
Investigator	$-\Delta U_R/m$	Cal. g. <sup>-1</sup>	$-\Delta U_B/m$	Cal. g. <sup>-1</sup>
Beckers <sup>1</sup>	3020.0	+0.7	3023.8	+0.7
Keffler	3018.6	-.7	3022.4	-.7
Roth and Beckers	3019.3	.0	3023.1	.0
This research	3019.3	.0	3023.1	.0
Mean	3019.3	± .35	3023.1	± .35

(1) Expressed in terms of the basic unity of energy, the above mean value for  $\Delta U_B/m$  is equal to 12,647.4 international joules per gram mass, with the "precision" and "accuracy" uncertainties being ±1.2 and ±3.2 joules per gram.

(2)  $(-\Delta U_B/m) - (-\Delta U_R/m) = 3.8 \text{ cal.}$ , or 15.9 joules, per gram.

(3) The uncertainties given in this table are the "precision" uncertainties.

Hippuric acid has been burned by at least three other workers, Stohmann and Schmidt,<sup>13</sup> Emery and Benedict,<sup>14</sup> and Berthelot and Andre.<sup>15</sup> Because of the low precision of these workers and also because of lack of experimental details, it will not be profitable to attempt to correct these results to a basis comparable with ours.

The values of  $-\Delta U_B/m$  for hippuric acid crystallized from water as given in Table II are for the standard bomb conditions. We also have calculated that the Washburn correction<sup>7</sup> is  $-0.078\%$ .

(13) Stohmann and Schmidt, *J. prakt. Chem.*, [2] **52**, 59 (1895).

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

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

Applying this correction, we find  $-\Delta U_R/m = 5624.2 \text{ cal. per gram at } 25.0^\circ$ . The temperature coefficient of the bomb reaction is  $-0.268 \text{ cal. g.}^{-1} \text{ degree}^{-1}$ . Using this datum and on the basis that the heat of combustion of benzoic acid is 6319.0 cal. at 25.0°, we calculate that  $-\Delta U_B/m = 5632.2 \text{ cal. g.}^{-1}$  under the standard bomb conditions at 20.0°.

In view of our findings in regard to the properties of hippuric acid, we propose and strongly urge its adoption as a standard for combustion calorimetry.

We have also calculated the "precision error" of our combustions on succinic acid and hippuric acid crystallized from water by the method given by Rossini.<sup>16</sup> Jessup and Green<sup>6</sup> have assigned an accuracy uncertainty of 0.023% to their determination of the heat of combustion of benzoic acids. From the results of nine calibration combustions, we have calculated that the calibration error is 0.0045%. The data on the combustion of succinic acid and hippuric acid lead to reaction errors of ±0.0089 and ±0.0043%, respectively. The latter is small because of the large number of good experiments. On this account we have chosen to use the mean deviation which is ±0.0088%. Hence we have assigned a precision error of ±0.009% to the combustion values of both of these substances. As far as we are aware, the only additional factor which will affect the absolute accuracy of these combustions is the presence of impurities. We feel that any error due to this cause will be extremely small, hence we have taken ±0.025% as the accuracy uncertainty for the heats of combustion of both of these compounds.

Finally we have collected in Table VI certain data derived from the combustion results on succinic acid and hippuric acid crystallized from

TABLE VI  
SUMMARY OF DERIVED DATA AT 25.0°

Substance	Succinic acid	Hippuric acid
Formula	C <sub>4</sub> H <sub>6</sub> O <sub>4</sub>	C <sub>8</sub> H <sub>9</sub> O <sub>3</sub> N
Molecular weight	118.087	179.168
Density	1.562	1.371
$-\Delta U_B$ , kcal. mole <sup>-1</sup>	356.99 ± 0.04 <sup>a</sup>	1008.46 ± 0.09 <sup>a</sup>
$-\Delta U_R$ , kcal. mole <sup>-1</sup>	356.54 ± 0.10 <sup>b</sup>	1007.67 ± 0.30 <sup>b</sup>
$-\Delta H_R$ , kcal. mole <sup>-1</sup>	356.24	1007.82
$-\Delta H_f$ , kcal. mole <sup>-1</sup>	225.66	147.85
$d\Delta U_R/dT$ , mole <sup>-1</sup> deg. cal.	-27	-44

<sup>a</sup> Precision uncertainty. <sup>b</sup> Accuracy uncertainty.

(16) Rossini, *Chem. Rev.*, **18**, 233 (1935).

water. In calculating the quantity  $-\Delta H_f^\circ$  we have used 68,313,<sup>17</sup> and 94,240,<sup>18</sup> respectively, as the heats of formation of liquid water and of gaseous carbon dioxide from graphite at 25.0°.

### Summary

Experimental data are reported for the heats of combustion of hippuric acid and succinic acid, and from these are calculated values for the heats of formation. These data are compared with existing values.

(17) Rossini, *Bur. Standards J. Research*, **6**, 34 (1931).

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

Hippuric acid is shown to be a substance whose properties fulfil the requirements for a bomb calorimetric standard, and it is proposed that hippuric acid (crystallized from water) be used as a secondary standard, for investigations on nitrogen-containing compounds. The value proposed for the heat of combustion of hippuric acid crystallized from water is 23,546.3 NBS international joules, or 5628.6 calories, per gram mass, under the standard conditions for the bomb process at 25°. The "precision" and "accuracy" uncertainties are, respectively,  $\pm 0.009$  and  $\pm 0.025\%$ .

PASADENA, CALIF.

RECEIVED NOVEMBER 1, 1937

[CONTRIBUTION FROM THE GATES AND CRELLIN LABORATORIES OF CHEMISTRY, CALIFORNIA INSTITUTE OF TECHNOLOGY, No. 636]

## The Magnetic Susceptibility of the Iron in Ferrohemo-globin

BY DONALD S. TAYLOR AND CHARLES D. CORYELL

### Introduction

The study of magnetic properties of hemoglobin derivatives and related compounds has been used as a method of elucidating their chemical structure.<sup>1</sup> It was found that the iron in oxyhemoglobin and carbonmonoxyhemoglobin is diamagnetic, and that the iron atom is bonded to six neighbors with covalent bonds. The iron atom in ferrohemo-globin<sup>2</sup> was found to have a magnetic moment about equal to that expected of a ferrous ion held in the structure by electrostatic bonds, although somewhat larger than usual for ionic ferrous complexes. It was suggested that the moment might be large because of interactions between the four heme groups of one molecule.

Interest in the absolute magnetic susceptibility of the iron of ferrohemo-globin is heightened by the fact that with the technique now in use hemoglobin concentrations can be determined as accurately by magnetic measurements as by the more tedious gasometric methods. Determinations have been made in this way by Coryell, Stitt, and Pauling<sup>1c</sup> in the study of magnetic properties and equilibria of ferrihemo-globin derivatives.

The possible presence in the blood of iron which does not combine with oxygen is a factor which should be considered in the determination of con-

centration and susceptibility of hemoglobin iron. Barkan<sup>3</sup> has shown that on acid denaturation a definite small fraction of the blood iron can be separated in the ionic form by ultrafiltration. He has called this fraction "leicht abspaltbares" iron and has postulated that it is formed from ferrous and ferric derivatives of hemoglobin with an altered heme structure which gives up iron more readily than ordinary hemoglobin does. By a comparison of the oxygen capacity and iron content of a number of blood samples (normal human blood, pathological human blood, and several animal bloods), Klumpp<sup>4</sup> has shown that there is about 5% (with a large spread of values) of the total iron in the blood which does not combine with oxygen. In the early magnetic investigations the possible effects of such iron were not taken into account, and they have been neglected in many other physico-chemical investigations.

It thus seemed desirable to redetermine the magnetic susceptibility of ferrohemo-globin with the superior technique now in use, taking into consideration the effect of the iron which does not combine with oxygen, and to ascertain how much variation there is from individual to individual and from species to species. It will be shown below that the value is constant within relatively small experimental error for individuals of one species,

(1) (a) L. Pauling and C. D. Coryell, *Proc. Nat. Acad. Sci.*, **22**, 159 (1936); (b) L. Pauling and C. D. Coryell, *ibid.*, **22**, 210 (1936); (c) C. D. Coryell, F. Stitt and L. Pauling, *THIS JOURNAL*, **59**, 633 (1937).

(2) The nomenclature used in this paper is that proposed by L. Pauling and C. D. Coryell.<sup>1b</sup>

(3) G. Barkan, (a) *Z. physiol. Chem.*, **171**, 179 (1927); (b) *ibid.*, **216**, 1 (1933); (c) G. Barkan and O. Schales, *ibid.*, **248**, 96 (1937). The last paper contains references to many other papers on the subject.

(4) T. G. Klumpp, *J. Clin. Investigation*, **14**, 351 (1935).