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

Thermal Data. V. The Heat Capacities, Entropies and Free Energies of Adenine, Hypoxanthine, Guanine, Xanthine, Uric Acid, Allantoin and Alloxan

BY ROBERT D. STIEHLER[1] AND HUGH M. HUFFMAN

In the preceding paper[2] in this series we have presented accurate combustion data on some purine and pyrimidine derivatives. In order that the data may be used to calculate the free energies of these compounds, we have also determined the heat capacities over a wide range of temperature. These data have been utilized to calculate the entropies, from which values and the heats of formation the free energies have been calculated.

Experimental.—In principle 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[3] so that only a brief account will be given.

In brief it consists in supplying a measured amount of heat electrically 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[4] (kindly given to us by Dr. K. K. Kelley).

Materials.—The adenine, guanine and hypoxanthine used in this investigation were prepared from nucleic acid. The xanthine and alloxan were commercial products obtained from Hoffman–LaRoche. The uric acid and allantoin were commercial preparations from Pfanstiehl and from Eastman, respectively. The preparation and purification of these compounds has been discussed adequately in the preceding paper[2] of this series. The purity of these compounds has been amply demonstrated by the combustion data. The adenine used in the heat capacity determinations crystallized in the form of long slender rods.

In view of the accuracy of the various 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 15° calorie[5] and with all weights reduced to a vacuum basis appear in Table I.

TABLE I

SPECIFIC HEATS PER GRAM OF SUBSTANCE

T, °K.	C_p	T, °K.	C_p	T, °K.	C_p
		Adenine, crystals			
88.3	0.0898	149.8	0.1347	230.3	0.1965
93.0	.0933	159.7	.1418	245.3	.2087
99.8	.0986	170.2	.1493	260.2	.2212
108.1	.1049	180.3	.1571	275.9	.2337
117.8	.1119	189.7	.1645	282.0	.2394
128.5	.1192	200.3	.1731	289.7	.2457
139.0	.1266	215.5	.1848	298.1	.2532
		Hypoxanthine, crystals			
85.3	0.0848	150.6	0.1298	230.2	0.1852
90.0	.0883	160.3	.1362	245.3	.1963
96.8	.0932	170.8	.1430	260.0	.2074
104.1	.0984	180.3	.1498	275.6	.2187
112.0	.1043	190.1	.1569	281.5	.2234
119.7	.1092	199.6	.1636	289.8	.2301
129.9	.1161	215.2	.1746	298.5	.2362
140.1	.1226				
		Guanine, crystals			
84.5	0.0820	150.8	0.1344	230.2	0.1965
89.0	.0856	160.2	.1413	245.2	.2078
95.7	.0910	171.0	.1498	260.4	.2202
103.0	.0969	180.8	.1573	276.1	.2326
111.4	.1036	190.2	.1650	281.4	.2360
120.5	.1107	199.4	.1724	288.0	.2415
130.7	.1187	215.2	.1850	296.7	.2482
141.0	.1266				
		Xanthine, crystals			
85.0	0.0825	150.7	0.1309	230.3	0.1878
89.7	.0861	160.6	.1377	245.4	.1985
96.4	.0912	170.8	.1447	260.3	.2091
103.7	.0967	180.6	.1516	275.5	.2205
110.6	.1022	190.3	.1588	281.3	.2248
118.9	.1083	200.3	.1661	289.2	.2307
129.2	.1157	215.5	.1771	298.5	.2378
140.2	.1234				

(1) National Research Fellow.

(2) Stiehler and Huffman, THIS JOURNAL, **57**, 1734 (1935).

(3) Parks, *ibid.*, **47**, 338 (1925).

(4) Giauque, Johnston and Kelley, *ibid.*, **49**, 2367 (1927).

(5) The factor 1.0004/4.185 was used to convert joules to calories.

TABLE I (*Concluded*)

T, °K.	C_p	T, °K.	C_p	T, °K.	C_p
		Uric acid, crystals			
85.9	0.0794	149.4	0.1285	232.6	0.1899
90.7	.0833	160.0	.1362	245.3	.1996
96.6	.0883	170.4	.1439	260.2	.2109
103.0	.0935	179.2	.1501	275.9	.2213
111.1	.0997	189.4	.1581	283.1	.2265
120.3	.1069	200.1	.1663	290.2	.2315
129.9	.1140	214.9	.1769	297.1	.2362
139.6	.1209				
		Allantoin, crystals			
84.6	0.0953	148.5	0.1504	229.6	0.2173
89.0	.1000	158.3	.1582	245.7	.2302
94.3	.1047	170.4	.1683	260.4	.2419
100.4	.1105	178.9	.1751	275.6	.2542
108.5	.1176	188.6	.1834	282.8	.2604
118.5	.1260	198.7	.1918	289.3	.2657
128.7	.1342	215.7	.2058	296.6	.2720
138.6	.1425				
		Alloxan, crystals			
85.5	0.0900	149.0	0.1453	229.6	0.2095
89.7	.0944	158.4	.1532	245.3	.2207
95.6	.0998	171.4	.1631	259.8	.2306
102.8	.1067	179.9	.1703	275.6	.2430
111.1	.1136	189.2	.1775	280.7	.2462
120.4	.1216	198.5	.1851	288.0	.2514
129.8	.1294	215.8	.1993	297.2	.2574
139.3	.1374				

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 extrapolation formula (for aromatic compounds) of Kelley, Parks and Huffman[6] for the increment from 0 to 90°K. and graphical integration between 90 and 298.1°K.

TABLE II

ENTROPIES OF THE COMPOUNDS PER MOLE

	S_{90}	$\Delta S_{90-298.1}$	$S_{298.1}$
Adenine	11.16	24.90	36.1
Hypoxanthine	10.89	23.87	34.8
Guanine	10.77	27.55	38.3
Xanthine	11.68	26.80	38.5
Uric acid	12.01	29.42	41.4
Allantoin	14.18	32.39	46.6
Alloxan	13.15	31.43	44.6

Since these compounds are considerably different both in composition and structure from those used in deriving the empirical extrapolation formula, the extrapolated portion of the entropy may have a greater error than estimated by Kelley, Parks and Huffman. However, for intercomparison among themselves this uncertainty will probably be largely canceled.

(6) Kelley, Parks and Huffman, *J. Phys. Chem.*, **33**, 1802 (1929).

The Free Energies.—We have also calculated the free energies of these compounds by the use of the fundamental thermodynamic equation $\Delta F = \Delta H - T\Delta S$. The essential data are given in Table III. We have used the heats of combustion as determined by us in the preceding paper.[2] The heats of formation have been calculated from the combustion values by use of the values 94,240[7] and 68,313[8] calories, respectively, for the heats of combustion of graphitic carbon and of hydrogen at 25°. The values of ΔS_{298}, the entropy of formation, were obtained by subtracting from the entropy of the compound the entropies of the elements contained therein. For the entropies of the elements we have used 1.36,[9] 31.23,[10] 45.78[11] and 49.03,[12] respectively, for graphitic carbon, hydrogen, nitrogen and oxygen.

TABLE III

THERMAL DATA AT 298.1

	Heat of comb. at const. π cal.	$\Delta H°_{298.1}$, cal.	$\Delta S_{298.1}$, E. U.	$\Delta F°_{298.1}$ cal.
Adenine	663,740	21,760	−163.2	70,420
Hypoxanthine	580,200	− 27,630	−150.5	17,250
Guanine	596,890	− 45,090	−185.6	10,220
Xanthine	516,020	− 91,810	−171.4	− 40,730
Uric acid	458,840	−148,980	−193.0	− 91,460
Allantoin	409,550	−172,350	−217.6	−107,470
Alloxan	273,580	−240,010	−191.7	−182,880

The error involved in the $T\Delta S$ term is probably of the order of 300 calories, due largely to uncertainties in the extrapolation of the specific heat curves from 90 to 0°K. With our new combustion data the accuracy of the free energies, for intercomparison purposes, we believe to be of the order of 300 to 600 calories.

When the free energy changes between certain of these compounds are examined some interesting facts are brought out. For example, in going from adenine to hypoxanthine and from guanine to xanthine, both of which involve the same type of deamination in different parts of the six membered ring, a decrease of 53,170 calories and 50,950 calories, respectively, is observed. Furthermore, in the changes adenine to guanine and hypoxanthine to xanthine to uric acid, all three reactions involving the addition of an oxygen

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

(8) Rossini, *Bur. Standards J. Res.*, **6**, 34 (1931).

(9) Jacobs and Parks, THIS JOURNAL, **56**, 1513 (1934).

(10) Giauque, *ibid.*, **52**, 4816 (1930).

(11) Giauque and Clayton, *ibid.*, **55**, 4875 (1933).

(12) Giauque and Johnston, *ibid.*, **51**, 2300 (1929).

atom and possibly a shift of a hydrogen with the corresponding opening of a double bond, the free energy decreases are, respectively, 60,200, 57,980 and 50,730 calories.

These changes indicate that, in the crystal at any rate, the bond energies are distinctly affected by their position in the compound.

Summary

1. The heat capacities of adenine, hypoxanthine, guanine, xanthine, uric acid, allantoin and alloxan have been determined over the temperature range 90 to 298.1°K.

2. From the heat capacities and an empirical extrapolation formula, the entropies at 298.1°K. have been calculated.

3. Using the entropies in conjunction with other data accurate free energies have been calculated for these compounds.

4. Some interesting relations between the free energies have been pointed out.

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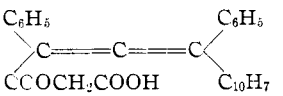
The Resolution of an Allenic Compound

BY E. P. KOHLER, J. T. WALKER AND M. TISHLER

For reasons too well known to require exposition it has long been desirable to know whether allenic compounds, in which at least one of the hydrogen atoms on each of the terminal carbon atoms of allene has been replaced by a substituent, can be obtained in optically active forms. The matter now seems to be definitely settled because Mills[1] has recently announced the formation of an optically active allene by an asymmetric degradation and we are able to report the production of optical opposites by the resolution of the acid



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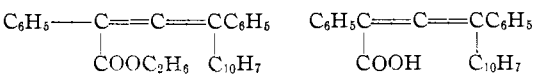
The reasons which ultimately led to the preparation of this acid for resolution and which justify its use for this purpose may be stated briefly as follows. Lapworth and Wechsler[2] in one of the earliest of the many attempts to prepare an allenic compound suitable for resolution[3] heated diphenyl naphthyl butyrophenone with phosphorus pentachloride and treated the mixture of products with alcohol and pyridine. They thus obtained a crystalline ester, and, by subsequent hydrolysis, a crystalline acid, to which with certain reservations they ascribed the allenic formulas



As these compounds can be obtained without great difficulty from material with which we are familiar we decided to employ them in our work.

In view of the reservations of Lapworth and Wechsler it was necessary at the outset to establish the allenic formulas with certainty. We began with the ester because it is easier to manipulate and we secured evidence that is conclusive. The composition, molecular weight and ethoxyl content are all in complete agreement with the formula. Oxidation of the ester with permanganate results in phenyl naphthyl ketone and an oil which is hydrolyzed to phenyl glyoxylic acid. On catalytic hydrogenation the ester adds two atoms of hydrogen rapidly—forming an intermediate reduction product which still reduces permanganate—and then less rapidly two more atoms of hydrogen to form a saturated ester. This evidence is conclusive because the ability to add four atoms of hydrogen excludes the possibility of any form of ring compound and the oxidation products show that the substituents are in their appointed places.

The acid has peculiar chemical and physical properties but its structure is as certain as that of the ester. It liberates a mole of gas from methylmagnesium iodide in the cold and it forms a silver salt from which ethyl iodide regenerates the ester. Although properly constituted, the acid does not lend itself to attempts at resolution because its salts appear to have no power to crystallize. As the esters crystallize well, the preparation of diastereomers by ester interchange with an optically

(1) Mills and Maitland, *Nature*, **135**, 994 (1935).

(2) Lapworth and Wechsler, *J. Chem. Soc.*, **97**, 38 (1910).

(3) For the most important references, see Freudenberg, "Stereochemie," p. 804.