Apparatus and Procedure.—The low temperature calorimeter has been previously described[1] and was used without modification.

A melamine sample of a little under one mole was employed. The calorimeter cooled with nitrogen refrigerant to the temperature of solid nitrogen and heat capacity measurements were made up to about 220°K. Dry Ice was then used as refrigerant for measurements to room temperature. Following this, the calorimeter was cooled with nitrogen and hydrogen refrigerant to the temperature of solid hydrogen and the interval 15 to 60°K. covered. The procedure with dicyandiamide was identical and a sample of a little over one mole was used.

The temperature and energy inputs were measured by means of a platinum–rhodium thermometer-heater having the laboratory designation R222. The resistance of this thermometer agreed with its original calibration[1] at the triple point of hydrogen and the freezing point of water. The calorie was taken to be 4.1833 international joules. The absolute temperature of the ice point was taken as 273.16°K.

Data and Discussion

Heat Capacity.—The experimental results for melamine and dicyandiamide are presented in Table I.

The accuracy of these results is estimated to be 0 2% above 35°K., 1% at 20°K., and 5% at 15°K.

Except for the values near 0°, all the results are corrected for the presence of water in the sample, as mentioned in a previous paragraph.

Entropy.—The contribution to the entropy of the heat capacity in the region 15 to 298.16°K. has been computed from the area under a large scale graphical plot of C_p/T *vs.* T. Around 15°, the heat capacity values of both compounds merge smoothly with the values of a Debye function of characteristic temperature $\theta = 175°$ and six degrees of freedom. The 0–15°K. contribution to the entropy is thus 0.20 e.u.

The entropy at 298.16°K. is for melamine 35.63 ± 0.03 e.u., and for dicyandiamide 30.90 ± 0.03 e.u.

Other Thermal Data.—The entropies of formation of melamine and dicyandiamide are calculated to be −199.39 and −125.78 e.u., respectively, employing entropy data for C, N_2 and H_2 from the tables of the National Bureau of Standards.[2] The heats of formation of melamine and dicyandiamide have been given by Salley and Gray[3] as −17.13 and 5.96 kcal./mole. Hence the free energies of formation of melamine and dicyandiamide are calculated to be 42.33 and 43.50 kcal./mole, respectively.

Acknowledgment.—The authors are indebted to the American Cyanamid Company for its support of this investigation.

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[1] R. W. Blue and J. F. G. Hicks, THIS JOURNAL, **59**, 1962 (1937).

[2] Selected Values of Properties of Hydrocarbons, National Bureau of Standards, Circular C461 (1947).

[3] D. J. Salley and J. B. Gray, THIS JOURNAL, **70**, 2650 (1948).

CAMBRIDGE, MASSACHUSETTS RECEIVED MAY 17, 1951

[CONTRIBUTION NO. 22 FROM THE THERMODYNAMICS LABORATORY, PETROLEUM EXPERIMENT STATION, BUREAU OF MINES]

2,2,3,3-Tetramethylbutane: Heat Capacity, Heats of Transition, Fusion and Sublimation, Vapor Pressure, Entropy and Thermodynamic Functions

BY D. W. SCOTT, D. R. DOUSLIN, M. E. GROSS, G. D. OLIVER AND H. M. HUFFMAN[1]

The heat capacity of solid 2,2,3,3-tetramethylbutane was studied between 12°K. and the melting point, 373.97 ± 0.05°K. The transition temperature (152.5 ± 0.2°K.), heat of transition (478 cal. mole^{-1}), and heat of fusion (1802 cal. mole^{-1}) were determined. The entropy of the solid at 298.16°K. was found to be 65.43 ± 0.15 cal. deg.$^{-1}$ mole^{-1}. The vapor pressure was measured from 0 to 65° and the following equation was selected to represent the data: $\log_{10} p = 7.92864 - 1709.428/(t + 233.634)$. The heat of sublimation calculated from the vapor pressure data is 10,365 ± 50 cal. mole^{-1}. The standard entropy of the vapor at 298.16°K. was found to be 93.05 ± 0.30 cal. deg.$^{-1}$ mole^{-1}. By utilizing the results of relatively detailed normal coördinate calculations as a guide in interpreting the spectra, a vibrational assignment adequate for purposes of thermodynamic computations was obtained. The average height of the potential barriers hindering internal rotation that is required to give agreement with the observed value of the entropy is 4700 cal. mole^{-1}. Using this value of the average barrier height, the vibrational assignment, and other pertinent molecular-structure data, the thermodynamic functions $-(F^0 - H_0^0)/T$, $H^0 - H_0^0$, S^0, and C_p^0 for 2,2,3,3-tetramethylbutane were computed for selected temperatures up to 1500°K.

Because of the interest of the petroleum industry in highly branched paraffin hydrocarbons, a study of certain thermodynamic properties of 2,2,3,3-tetramethylbutane (hexamethylethane) was undertaken by the Thermodynamics Laboratory of the Petroleum and Natural Gas Branch of the Bureau of Mines.

The molecule of 2,2,3,3-tetramethylbutane is the most compact and most highly symmetrical of the isomeric octane molecules and is the only one of these that does not have rotational isomers. By virtue of this high molecular symmetry and lack of rotational isomers, the detailed methods of statistical mechanics could be used in a particularly straightforward manner to calculate the thermodynamic functions of 2,2,3,3-tetramethylbutane. Of the molecular-structure parameters required for such calculations, the moments of inertia and vibrational frequencies could be obtained from available electron-diffraction and spectroscopic data, respectively. However, information about the potential barriers hindering internal rotation required an experimental value of some thermodynamic property such as a value of the entropy of the ideal gas obtained from third-law studies.

This paper presents (a) the results of calorimetric and vapor-pressure measurements by which the necessary entropy value was obtained and (b) the calculated thermodynamic functions made possible by these experimental results.

Relative to most hydrocarbons of about the same molecular weight, 2,2,3,3-tetramethylbutane has a high melting point (100.81°) and an unusually

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[1] Deceased.

short liquid range (less than 6°). The calorimetric studies of this research were extended above room temperature to include the melting point and short liquid range of the compound.

Experimental

The Material.—The sample of 2,2,3,3-tetramethylbutane used for the experimental measurements was prepared in the Research Laboratories of the Ethyl Corporation and supplied through the courtesy of Dr. George Calingaert. The method of purification of the material has been described by Calingaert, Soroos, Hnizda and Shapiro.[2]

Calorimetric Measurements.—The measurements of heat capacity, heats of transition and fusion, transition temperature, and melting point were made in the apparatus described by Ruehrwein and Huffman.[3] The sample (33.426 g.) was contained in a sealed platinum calorimeter having heat-distributing disks of gold. To fill the calorimeter, it was connected to a glass transfer system through a small monel tube and a metal-to-glass seal, and the sample was distilled in under its own vapor pressure. During the latter operation the transfer system and calorimeter were maintained above the melting point of the compound (100.81°) to prevent plugging. After the calorimeter had been filled, the monel tube was pinched off and a drop of soft solder was applied to the tip of the tube. A vacuum-tight seal was not obtained the first time, and it was necessary to reconnect the calorimeter to the vacuum system, pump out the air, and seal off a second time. It is believed that the trace of water that may have been introduced by the initial failure to obtain a tight seal was too little to have any significant effect on the experimental measurements.

The method used for measuring the heat capacity was, very briefly, as follows: a measured amount of electrical energy was supplied to the calorimeter, and at all times the temperature of the environment was maintained at that of the calorimeter to prevent heat interchange. The initial and final temperatures of the calorimeter were measured by means of a platinum resistance thermometer. The heat capacity of the calorimeter plus contents was obtained from the measured energy input and observed temperature rise. Subtracting the heat capacity of the calorimeter, determined by a previous calibration with the calorimeter empty, gave the net heat capacity of the sample. The precision of the measurements was, in general, better than 0.1%, and above 30°K. it is believed that the accuracy uncertainty should not be greater than 0.2%. The energy measurements were made in terms of the international joule and were converted to calories by dividing by 4.1833. At the higher temperatures a correction was applied for vaporization into the gas space. This correction was significant only above 280°K., and reached a maximum value of 0.44% for the measurement immediately below the melting point. The results of the heat capacity measurements are given in Table I.

Crystalline 2,2,3,3-tetramethylbutane has a transition between a form stable at low temperatures (α-form) and a form stable at higher temperatures (β-form). The transition temperature was studied as a function of the per cent. transposed into the β-form with the following results: 23.5%, 152.38°K.; 52.7%, 152.44°K.; and 91.5%, 152.48°K. The value taken for the transition temperature is 152.5 ± 0.2°K. Duplicate determinations of the heat of transition gave the values 477.6 and 478.2 cal. mole^{-1}.

The pronounced change of the transition temperature with increasing amounts transposed suggests the presence of impurity which can form solid solutions. The fact that Parks, Huffman and Thomas,[4] in studies on a sample of presumably lower purity, observed a significantly lower transition temperature (148.1°K.) and higher "pretransition" heat capacities lends support to this explanation. Presumably, in the β-form of 2,2,3,3-tetramethylbutane the nearly spherical molecules have considerable rotational freedom. The loose type of crystal structure associated with such rotation in the solid is known to favor the formation of solid solutions. In fact, Smittenberg, Hoog and Henkes[5] have found that 2,2,3,3-tetramethylbutane forms a continuous series of solid solutions with 2,2,3-trimethylbutane.

Table I

Molal Heat Capacity of 2,2,3,3-Tetramethylbutane

0°C. = 273.16°K.; mol. wt. 114.224

T,[a] °K.	$C_{sat.}$, cal. deg.$^{-1}$	T, °K.	$C_{sat.}$, cal. deg.$^{-1}$	T, °K.	$C_{sat.}$, cal. deg.$^{-1}$
α-Crystals		80.38	17.257	213.92	43.737
12.12	1.951	83.07	17.797	223.21	44.936
12.91	2.259	86.75	18.535	233.94	46.809
13.45	2.436	91.51	19.461	244.36	48.377
14.09	2.710	93.61	19.868	254.49	49.966
15.21	3.096	100.09	21.173	264.37	51.478
15.98	3.342	100.35	21.238	274.00	52.987
17.57	3.957	101.80	21.522	283.40	54.467
18.44	4.316	109.18	23.114	292.60	55.916
20.74	5.163	109.44	23.165	301.60	57.27
21.41	5.423	118.17	25.178	303.40	57.62
24.32	6.394	122.50	26.200	313.42	59.33
24.37	6.411	126.84	27.344	316.88	59.87
24.92	6.612	130.05	28.264	323.20	60.99
27.92	7.477	135.36	30.013	326.89	61.60
28.21	7.569	137.16	30.645	332.77	62.63
28.69	7.716	143.28	33.437	341.00	64.01
31.97	8.543	β-Crystals		342.43	64.32
36.52	9.551	159.67	35.163	350.47	65.75
41.72	10.535	160.74	35.317	358.97	67.42
47.32	11.509	166.87	36.250	359.60	67.58
53.22	12.510	173.35	37.264	362.97	68.33
55.48	12.893	179.00	38.139	365.79	68.78
60.73	13.761	181.13	38.532	Liquid	
61.08	13.809	190.57	40.050	375.41	66.94
67.48	14.902	194.97	40.757	377.09	67.24
74.85	16.198	200.40	41.638	377.99	67.31
		204.30	42.288		

[a] All temperatures listed are the mean temperatures of the individual measurements. The temperature increments employed were varied from about 1° at the lowest temperatures to about 10° at the highest temperatures.

Results that likewise could be interpreted by assuming the presence of solid-soluble impurity were obtained in studies of the melting point as a function of the percentage melted. In these experiments, which were carried out in the manner described previously,[6] the approach to equilibrium was extremely slow, and it is doubtful if it was ever attained. Freezing points, obtained by partially freezing the liquid, interrupting the cooling, and allowing thermal equilibrium to be approached, were several hundredths of a degree lower for the same fraction in the liquid state, than melting points obtained by partially melting the solid and allowing thermal equilibrium to be approached from the opposite direction. Some typical values of melting point as a function of the percentage melted follow: Series I—6.8%, 373.791°; 24.89%, 373.860°; 49.72%, 373.899°; Series II—70.57%, 373.901°; 90.84%, 373.933°K. These data do not yield a linear plot of temperature *vs.* reciprocal of fraction melted such as is obtained for ideal solutions and solid-insoluble impurities. Furthermore, the points for the two series do not lie on a smooth curve, probably because of failure to attain equilibrium, as mentioned above. Calculating the concentration of impurity in the sample in the conventional manner, using the temperatures for 49.72 and 90.84% melted, gives 0.024 mole per cent. Because of the likelihood of solid-soluble impurity, this value is quite arbitrary and is probably lower than the true concentration of impurity present. Extrapolation of temperature to the melting point of the pure material is necessarily somewhat

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(2) G. Calingaert, H. Soroos, V. Hnizda and H. Shapiro, This Journal, **66**, 1389 (1944).

(3) R. A. Ruehrwein and H. M. Huffman, *ibid.*, **65**, 1620 (1943).

(4) G. S. Parks, H. M. Huffman and S. B. Thomas, *ibid.*, **52**, 1032 (1930).

(5) J. Smittenberg, H. Hoog and R. A. Henkes, *ibid.*, **60**, 17 (1938).

(6) S. S. Todd, G. D. Oliver and H. M. Huffman, *ibid.*, **69**, 1519 (1947).

uncertain; 373.97 ± 0.05°K. was adopted for the melting point in the present investigation.

Two determinations of the heat of fusion gave 1802.0 and 1802.5 cal. mole^{-1}. The cryoscopic constant computed from the observed heat of fusion is 0.00648 deg.$^{-1}$.

At the time these measurements were made (April, 1944 and March, 1948) the results of the dilatometric studies of Seyer, Bennett and Williams[7] were unknown to the authors. Seyer and co-workers found that a third crystalline modification of 2,2,3,3-tetramethylbutane (γ-form) is the stable form above 347.41°K. They observed that the β-form persists in a metastable condition between the β–γ transition temperature and the melting point, and that under the conditions of their experiments nucleation and subsequent transformation to the γ-form occurred only after freezing the liquid and then cooling extremely slowly to about 1° below the melting point. The γ-form was not obtained in the present investigation, and the values reported here for the melting point, heat of fusion, cryoscopic constant, and solid heat capacity above 347.41°K. are for the β-form in the metastable state.

TABLE II

VAPOR PRESSURE OF 2,2,3,3-TETRAMETHYLBUTANE (SOLID)

t, °C.	0.00	15.00	20.00	25.00	30.00	35.00
p(obsd.), I	4.12	11.25	15.45	20.89	27.93	36.77
mm. II	4.07	11.25	15.35	20.81	27.76	36.70
p(calcd.), mm.	4.09	11.31	15.45	20.85	27.83	36.75
t, °C.	40.00	45.00	50.00	55.00	60.00	65.00
p(obsd.), I	48.11	62.25	79.93	101.53	128.04	160.37
mm. II	48.01	62.10	79.70	101.30	127.78	159.88
p(calcd.), mm.	48.03	62.17	79.76	101.43	127.94	160.13

TABLE III

ENTROPY OF 2,2,3,3-TETRAMETHYLBUTANE, CAL. DEG.$^{-1}$ MOLE^{-1}

0–12°K.	Debye extrapolation	0.712	
12–152.5°	Solid (α-form), graphical $\int C_{sat.}\,d\ln T$	31.956	
152.5°	Transition, 477.9/152.5	3.134	
152.5–298.16°	Solid (β-form), graphical $\int C_{sat.}\,d\ln T$	29.627	
Entropy of solid at 298.16°K.			65.43 ± 0.15
298.16–373.97°	Solid (β-form), graphical $\int C_{sat.}\,d\ln T$	14.286	
373.97°	Fusion, 1802.2/373.97	4.819	
Entropy of liquid at 373.97°K.		84.53 ± 0.20	
298.16°	Sublimation, 10,365/298.16		34.76
298.16°	Compression, $R\ln(p/760)$		−7.15
298.16°	Gas imperfection		0.01
Entropy of ideal gas at 1 atm. and 298.16°K.			93.05 ± 0.30

Vapor Pressure Measurements.—The vapor pressure of 2,2,3,3-tetramethylbutane in the solid state was studied by a static method, using the apparatus and methods described in a prior publication from this Laboratory.[8] The results of the measurements are given in Table II. The data of series I were obtained after subliming material out of the sample container until about four-fifths of the original sample remained; the data of series II were obtained after subliming out additional material until only about one-fifth of the original sample remained. Comparison of the two series shows that the observed vapor pressures were decreased a few-tenths of a per cent. by subliming out the additional quantity of material. This behavior indicates a small amount of impurity of different volatility. The presence of such impurity introduces some uncertainty into the experimental values of the vapor pressure but has little effect on the value of the heat of sublimation calculated from the vapor pressure data, since both series of measurements give almost identical values of d log p/d($1/T$).

The Antoine equation

$$\log_{10} p = 7.92864 - 1709.428/(t + 233.634) \quad (1)$$

the constants of which were determined by a least-squares adjustment,[9] was fitted to the vapor pressure data. Values of the vapor pressure calculated by means of equation (1) are listed in Table II for comparison with the observed values.

The heat of sublimation at 25° was calculated from the vapor pressure data by means of the Clapeyron equation. The Berthelot equation of state with the critical constants, p_c = 24.5 atm. and t_c = 270.8°C.,[10] was used to calculate the molal volume of the vapor. The value obtained for the heat of sublimation is 10,365 cal. mole^{-1}, with an estimated uncertainty of ±50 cal. mole^{-1}. Osborne and Ginnings[11] determined the heat of sublimation calorimetrically and obtained the value 10,256 ± 205 cal. mole^{-1}, their large uncertainty arising from experimental difficulties in using a calorimeter designed primarily for measuring heats of vaporization of liquids.

Entropy.—The entropy of 2,2,3,3-tetramethylbutane was computed from the calorimetric and vapor pressure data. A summary of the computation is given in Table III. For extrapolating the heat capacity below 12°K. a Debye function for five degrees of freedom with θ = 83.72° was used. The Berthelot equation of state was employed in computing the correction for gas imperfection.

Thermodynamic Functions

Vibrational Assignment.—In order to utilize the experimental entropy value to obtain information about the potential barriers hindering internal rotation in 2,2,2,3,-tetramethylbutane, it was necessary to have a vibrational assignment for the molecule. Interpretation of the available spectroscopic data led to an assignment which was adequate for that purpose and for the subsequent calculation of thermodynamic functions.

The Raman and infrared spectra of 2,2,3,3-tetramethylbutane have been studied by a number of investigators.[12] The spectroscopic data used in making the vibrational assignment are listed in

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[7] Wm. F. Seyer, R. B. Bennett and F. C. Williams, THIS JOURNAL, **71**, 3447 (1949).

[8] G. Waddington, J. W. Knowlton, D. W. Scott, G. D. Oliver, S. S. Todd, W. N. Hubbard, J. C. Smith and H. M. Huffman, *ibid.*, **71**, 797 (1949).

[9] C. B. Willingham, W. J. Taylor, J. M. Pignocco and F. D. Rossini, *J. Research Natl. Bur. Standards*, **35**, 219 (1945).

[10] W. A. Felsing, A. M. Cuellar and W. M. Newton, THIS JOURNAL, **69**, 1972 (1947).

[11] N. S. Osborne and D. C. Ginnings, *J. Research Natl. Bur. Standards*, **39**, 453 (1947).

[12] (a) P. Lambert and J. Lecomte, *Ann. phys.*, **10**, 503 (1938) (infrared to 15 microns); (b) P. A. Bazhulin, M. F. Bokshtein, A. L. Liberman, M. Yu. Lukina, E. I. Margolis, O. P. Solovova and B. A. Kazanskii, *Bull. acad. sci. U. R. S. S., Classe sci. chim.*, 198 (1943) (Raman in CCl_4 soln.); (c) R. A. Oetjen and H. M. Randall, *Rev. Mod. Phys.*, **16**, 265 (1944) (infrared of vapor to 15 microns); (d) American Petroleum Institute Research Project 44 at the National Bureau of Standards, Catalog of Infrared Spectrograms, Serial No. 444 (infrared to 15 microns for CS_2 and CCl_4 solns.); (e) F. F. Cleveland, J. E. Lamport and R. W. Mitchell, *J. Chem. Phys.*, **18**, 1320 (1950) (Raman with polarizations and infrared to 25 microns for solns. in benzene, CCl_4 and CS_2).

Table IV. Except as indicated by footnotes, only strong Raman lines and infrared bands that have been observed in every investigation of the particular spectrum are included in the table. As an aid in interpreting these spectroscopic data, normal coördinate calculations of the lower frequencies were carried out. Wilson's G–F matrix method[13] was employed and the higher C–H stretching and methyl bending frequencies were factored out at once. The force constants were selected to give exact agreement with five frequencies of 2,2-dimethylpropane (733, A_1; 335, E; 414, 926 and 1257 cm.$^{-1}$, T_2) when the calculations for that molecule were carried out in an analogous manner.

TABLE IV

SPECTRA OF 2,2,3,3-TETRAMETHYLBUTANE BELOW 1300 CM.$^{-1}$

Abbreviations used: p, polarized; d, depolarized; ‖, parallel band contour.

Raman, cm.$^{-1}$	Infrared, cm.$^{-1}$	Raman cm.$^{-1}$	Infrared, cm.$^{-1}$
277^a		877 p	
385d		923 p	
431^a			930
449^a			998
479d		1043	
507^a	*ca.* 505^b		1182
647p	796‖		*ca.* 1235^c
		1238 p	
819d		1256 d	

[a] Reported only in ref. 12b. [b] Very weak band in the published infrared spectrogram of ref. 12e. [c] Reported only in ref. 12d.

TABLE V

CALCULATED[a] AND ASSIGNED FREQUENCIES OF 2,2,3,3-TETRAMETHYLBUTANE, CM.$^{-1}$; SYMMETRY: D_{3d}

	Calcd.	Assigned		Calcd.	Assigned
		(2950)^b (2)			(2950) (2)
		(1465)			(1465)
A_{1g}		(1380)	A_{2u}		(1380)
Raman	1304	1238	Infrared	1138	1182
polarized	904	900^c	parallel	817	796
	652	647		480	507^d
	284	277			
A_{2g}		(2950)	A_{1u}		(2950)
Inactive		(1465)	Inactive		(1465)
	971	971^e		971	971^e
		(2950) (3)			(2950) (3)
		(1465) (2)			(1465) (2)
E_g		(1380)	E_u		(1380)
Raman	1262	1256	Infrared	1224	1235
depolarized	980	1043	perpendicular	978	998
	936	819		945	930
	492	479		377	449^d
	309	385		203	216^f

[a] Using the following force constants: C–C stretching, 4.758×10^5 dynes/cm.; C–C–H bending, 0.616×10^{-11} erg/radian^2; C–C–C bending, 1.026×10^{-11} erg/radian^2; interaction between the angle bending and the stretching of a C–C bond of a C–C–C group, 0.494×10^{-3} dyne/radian; and interaction between the bending of two C–C–C angles having a vertex and side in common, 0.048×10^{-11} erg/radian^2. [b] Parentheses denote average or conventional values. [c] Mean of the 877–923 cm.$^{-1}$ doublet resulting from Fermi resonance with the first harmonic of the 449 cm.$^{-1}$ frequency. [d] Frequencies observed in the Raman spectrum in violation of the strict vapor-phase selection rules. [e] Inactive frequency for which the calculated value was used. [f] Assigned by interpreting the weak Raman line at 431 cm.$^{-1}$ as the first harmonic of this frequency.

Table V summarizes the results of the normal coordinate calculations and lists the complete frequency assignment used for computing the thermodynamic functions. The agreement between observed and calculated frequencies is as good as is to be expected in view of the greatly oversimplified potential function used in the normal coördinate treatment. From the standpoint of thermodynamic calculations, the most uncertain feature of the vibrational assignment is the value of the lowest E_u frequency, which has not been observed directly and was assigned by interpreting a weak Raman line as its first harmonic. However, as the following considerations show, the uncertainty in the value of this frequency is not of serious consequence. In using the experimental value of the entropy to evaluate the average height of the potential barriers hindering internal rotation, the use of too low a value for a skeletal bending frequency will result in too high a value for the average barrier height and *vice versa.* When computing thermodynamic functions, an error in a skeletal bending frequency will then be largely compensated by the concomitant error in the average barrier height.

Moments and Reduced Moments of Inertia.—Internal rotation in the 2,2,3,3-tetramethylbutane molecule is a case of compound rotation. The moments and reduced moments of inertia were therefore calculated by the methods developed for that case by Kilpatrick and Pitzer.[14] The electron-diffraction values of 1.58 Å. for the central C–C distance and 1.54 Å. for the other six C–C distances[15] were used. A C–H distance of 1.09 Å. was assumed, and all bond angles were taken to be tetrahedral. The three principal moments of inertia for over-all rotation were calculated to be $I_A = 3.669 \times 10^{-38}$ g. cm.^{2} and $I_B = I_C = 4.868 \times 10^{-38}$ g. cm.^{2}. The off-diagonal elements in the reduced internal rotational matrix (D) were found to be small, and therefore the internal rotations could be treated independently, using the diagonal elements as reduced moments of inertia. These were 5.243×10^{-40} g. cm.^{2} for each of the six methyl rotations and 91.73×10^{-40} g. cm.^{2} for rotation about the central C–C bond.

Barriers to Internal Rotation.—In the 2,2,3,3-tetramethylbutane molecule the six potential barriers hindering rotation of the methyl groups are equivalent; the potential barrier hindering rotation about the central C–C bond is, of course, different from these. However, for calculating thermodynamic functions it is justifiable to assume that all seven potential barriers are the same and to use a single average value for the height of each. The difference between the methyl and skeletal barriers is probably small and less significant than interactions between the different internal rotations, which must also be neglected in the thermodynamic calculations.

The value of the average barrier height that is required to give agreement with the experimental value of the entropy is 4700 cal. mole^{-1}. This may be compared with the average barrier heights

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[13] E. B. Wilson, Jr., (a) *J. Chem. Phys.*, **7**, 1047 (1939); (b) *ibid.*, **9**, 76 (1941).

[14] J. E. Kilpatrick and K. S. Pitzer, *ibid.*, **17**, 1064 (1949).

[15] S. H. Bauer and J. Y. Beach, THIS JOURNAL, **64**, 1142 (1942).

of two other highly branched hydrocarbons, 2,2-dimethylpropane (4300 cal. mole^{-1})[16] and 2,2-dimethylbutane (4375 cal. mole^{-1}).[17]

Thermodynamic Functions.—The functions $-(F^0 - H_0^0)/T$, $H^0 - H_0^0$, S^0 and C_p^0 for 2,2,3,3-tetramethylbutane were computed for selected temperatures up to 1500°K., using the vibrational assignment, moments and reduced moments of inertia, and value of the average barrier height discussed in previous sections. The 1949 atomic weights and the values of the fundamental constants given by Wagman, *et al.*,[18] were used in all computations of this paper. The calculated thermodynamic functions are presented in Table VI. For reasons of internal consistency, all tabulated values are given to four digits, although in some places in the table the retention of the last digit is not justified by the accuracy of the molecular-structure parameters used in the calculations or by the reliability of the approximations employed.

TABLE VI

THERMODYNAMIC FUNCTIONS OF 2,2,3,3-TETRAMETHYLBUTANE

T, °K.	$-(F^0 - H_0^0)/T$, cal. deg.$^{-1}$ mole^{-1}	$H^0 - H_0^0$ kcal. mole^{-1}	S^0, cal. deg.$^{-1}$ mole^{-1}	C_p^0, cal. deg.$^{-1}$ mole^{-1}
298.16	67.97	7.482	93.06	46.03
300	68.12	7.565	93.34	46.29
400	76.33	12.88	108.5	59.88
500	84.27	19.48	123.2	71.76
600	91.94	27.17	137.2	81.52
700	99.35	35.73	150.4	89.52
800	106.5	45.02	162.8	96.18
900	113.4	54.92	174.4	101.8
1000	120.1	65.34	185.4	106.6
1100	126.5	76.21	195.8	110.8
1200	132.7	87.49	205.6	114.4
1300	138.7	99.04	214.9	117.5
1400	144.4	110.9	223.7	120.1
1500	150.0	123.1	232.0	122.5

BARTLESVILLE, OKLAHOMA RECEIVED JULY 17, 1951

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[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF NORTHWESTERN UNIVERSITY]

The Thermodynamics of Metallo–Protein Combinations. Comparison of Copper Complexes with Natural Proteins

BY HAROLD A. FIESS AND IRVING M. KLOTZ

The affinity of each of five proteins for cupric ion in acetate buffer at *p*H 6.5 has been found to decrease in the following order: α-casein > β-casein > bovine serum albumin > β-lactoglobulin > lysozyme. Relative affinity for copper parallels the isoelectric point of these proteins. The variation in binding with copper concentration is less than might be expected on superficial examination of the electrostatic factors involved, but more careful consideration indicates some reasons for the discrepancy. Binding of copper by α-casein exhibits no temperature dependence between 0° and 25°. Furthermore heating of the protein to 90° for 1 hour does not reduce its affinity for copper. These characteristics, as well as the interactions of copper with the polypeptides of glutamic acid and of lysine, emphasize the importance of electrostatic factors in the formation of copper–protein complexes.

Introduction

Detailed thermodynamic studies of copper complexes with crystallized bovine serum albumin have been reported recently.[1,2] In these papers the effects of environmental factors, such as *p*H, type of buffer, and temperature, on the stability of copper–albumin complexes have been described. Further investigations comparing the affinities for copper of a number of native proteins and related polypeptides are described in the present paper.

Experimental

Quantitative binding studies were made by the equilibrium–dialysis technique described previously,[1] except that smaller volumes of solution were used.[3] The protein concentration was 0.5% in all binding experiments.

Analyses for cupric ion concentrations were made by the cuprethol method of Woelfel.[4] This method gave accurate results for copper in the presence of acetate, citrate, phosphate or borate ions as well as in the presence of any of the proteins used in this study. An acetate buffer of *p*H 5, as recommended by Woelfel, was used in most analyses. In the analysis of casein-containing solutions, however, precipitation occurred at this *p*H and therefore an acetate or citrate buffer of *p*H 6.5 was used.

Absorption spectra were obtained with a Beckman spectrophotometer, model DU, using one-centimeter cells. Molecular extinction coefficients, ϵ, were calculated from the equation

$$\log_{10} \frac{I_0}{I} = \epsilon c d$$

where I_0 is the intensity of the light emerging from the solvent, I is the intensity of the light emerging from the solution, c is the molar concentration of solute, and d is the thickness of the absorption cell in centimeters.

Crystallized bovine serum albumin and lysozyme were obtained from Armour and Co. Samples of crystallized β-lactoglobulin were kindly supplied by Dr. L. Lachat of Armour and Co. The lactoglobulin gave signs of the presence of trace metals in blank tests with cuprethol reagent. This protein was dialyzed, therefore, against 0.02 *M* sodium citrate to remove the trace metals and then against water to remove the citrate. The purified protein was lyophilized.

α-Casein and β-casein were prepared from unpasteurized skim milk by the procedure of Warner.[5] All-glass equipment was used to prevent contamination by trace metals. The fractionated caseins gave electrophoretic patterns similar to those described by Warner.[5]

A sample of natural polyglutamic acid was kindly supplied by Dr. H. Fraenkel-Conrat. Polylysine hydroiodide was a gift from Dr. E. Katchalski.

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