human serum albumin with 54 amino groups guanidinated. (Assuming this modification has no effect on the extinction except that due to the increase in molecular weight mentioned above, the extinction of normal human serum albumin should have been decreased from 5.3 to 5.1 following guanidination.) The optical rotation $[\alpha]^{25}_{5461}$ of the same guanidinated product was $-65°$, a significant decrease from the value of $-78°$ found for normal human serum albumin.

The homogeneity of guanidinated serum albumin may be considered both as evidenced by the usual physical chemical criteria of homogeneity and as implied by the stoichiometry of the reaction. Assuming that one started with a pure protein (one component) and reacted all the groups of one type in the protein in the same manner, the final product should be homogeneous, although the intermediates might be very heterogeneous.[20]

Since it has not proven possible to convert the amino groups of serum albumin quantitatively to guanidino groups, heterogeneity in the fine structure of the products cannot be excluded. However, the few remaining amino groups in the most guanidinated products would appear to be of a very different reactivity from the rest, and therefore the heterogeneity must be of a much lower order than that predicted on a purely statistical basis.

Unfortunately serum albumin itself does not fulfill the phase-rule requirements of a pure substance (*i. e.*, constant solubility with variation of the amount of saturating body). Consequently, guanidinated albumin could not be expected to meet this most rigorous test. However, when analyzed in the ultracentrifuge, in the electrophoresis apparatus, and in the viscosimeter, guanidinated albumins appear no less homogeneous than the starting material, a conclusion which is markedly strengthened by the crystallizability of highly guanidinated products.

Summary

1. Human serum albumin reacts with O-methyl isourea with the formation of guanidinated derivatives.

2. The preparation and properties of methyl isourea acid sulfate are described.

3. The reaction appears specific for the amino groups of serum albumin, largely converting the lysine residues into homo-arginine residues.

4. By controlling the *p*H, the proportion of the 68 amino groups converted to guanidino groups can be varied from less than 10 below *p*H 9 to more than 50 above *p*H 10.

5. The preparation and properties of a crystallized guanidinated derivative of human serum albumin are described.

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[20] Herriott and Northrop would appear to have prepared a homogeneous acetyl derivative of pepsin. They found that with limited amounts of ketene the four amino groups could be quantitatively and specifically covered: *J. Gen. Physiol.*, **18**, 35 (1934).

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF THE UNIVERSITY OF ILLINOIS]

The Determination of the Decomposition Pressures of Certain 1,10-Phenanthroline Hydrates

BY JAMES S. FRITZ,[1] F. WM. CAGLE, JR.,[2] AND G. FREDERICK SMITH

The recognition of the hydrogen bond and appreciation of its extent were due to Latimer and Rodebush,[3] who used this concept to explain abnormally high dielectric constants for water and hydrogen fluoride, the small ionization of ammonium hydroxide and the dimerization of certain carboxylic acids. Since then the hydrogen bond (or hydrogen bridge) concept has been used to explain qualitatively many abnormal properties of organic compounds.[4] The instances in which the strength of a hydrogen bond has been estimated quantitatively are, however, not so numerous. From the heats of dimerization, the O—H...O bond strength in formic acid has been estimated to be 7.2 cal./mole[5] and in acetic acid to be 7.25 cal./mole.[6] For similar bonds in *o*-hydroxybenzoic acid and benzoic acid, values of 4.7 and 4.3 cal./mole, respectively, have been found.[7] The strength of the hydrogen bonds in methanol and ethanol has been estimated to be about 6.2 cal./mole.

1,10-Phenanthroline as well as the 5-bromo and 5-methyl derivatives form monohydrates. Infrared data indicate that the water molecule is held through two hydrogen bonds to the ring nitrogens.[8] These hydrates are definite chemical entities with sharp and reproducible melting points. When 1,10-phenanthroline monohydrate is heated, it dissociates reversibly and rapidly into 1,10-phenanthroline and water.

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[3] Latimer and Rodebush, THIS JOURNAL, **42**, 1419 (1920).

[4] Huggins, *J. Org. Chem.*, **1**, 407 (1936).

[5] Halford, *J. Chem. Phys.*, **10**, 582 (1942).

[6] Ritter and Simons, THIS JOURNAL, **67**, 757 (1945); Halford, *J. Chem. Phys.*, **9**, 859 (1941); **10**, 582 (1942).

[7] Pauling, "The Nature of the Chemical Bond," Cornell University Press, Ithaca, N. Y., 1944, pp. 288–289.

[8] Smith and Richter, "Phenanthroline and Substituted Phenanthroline Indicators," G. Frederick Smith Chemical Company, Columbus, Ohio, 1944.

The purpose of this investigation is to measure the dissociation pressure of certain 1,10-phenanthroline monohydrates and hence the equilibrium constants for reactions of the type

$$C_{12}H_8N_2 \cdot H_2O(s) = C_{12}H_8N_2(s) + H_2O(g)$$

From these data, measurements of the strengths of the hydrogen bonds readily can be obtained.

Materials

1,10-Phenanthroline.—The reagent grade was further purified by recrystallization from water and dried for several hours in a desiccator. A portion of the hydrate so obtained was converted to the anhydrous compound by fusion. The melting points are: hydrate 99-100°, anhydrous 117°.

5-Bromo-1,10-phenanthroline.—A commerical sample was further purified by recrystallization from moist benzene and the anhydrous material obtained as above. The melting points are: hydrate 84°, anhydrous 118°.

5-Methyl-1,10-phenanthroline.—Purification was effected by two recrystallizations from benzene-petroleum ether mixtures. In order to effect the complete removal of organic solvents, the hydrate was prepared by fusion of the original sample followed by solution in water and careful recrystallization. The anhydrous compound was formed in the usual manner. The melting points are: hydrate 67°, anhydrous 114°.

Apparatus.—The apparatus is shown in Fig. 1. The essential features of this apparatus are:

1. A Pyrex sickle gage whose use and manufacture have been described by Phipps and co-workers.[9]
2. A thermostat consisting of a Dewar-like Pyrex vessel, the temperature of which could be adjusted by the ebullition of a suitable organic liquid contained between the walls.
3. A mercury manometer the pressure differential of which was observed by a suitably disposed cathetometer.

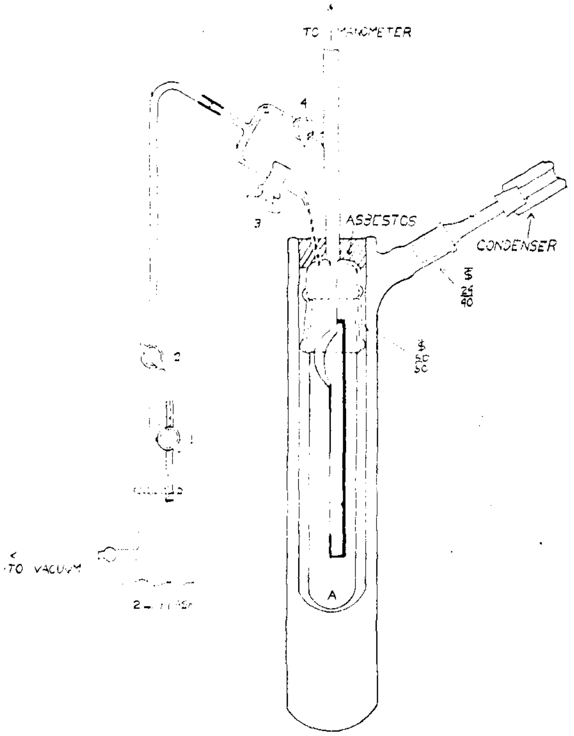

Fig. 1.—Phipps sickle gage.

Experimental Procedure

Approximately 5 g. of the hydrate is mixed intimately with 3 g. of the anhydrous material, the latter being added to react with any excess moisture held by the hydrate and to insure equilibrium at all times. The sample is introduced into tube A of the apparatus shown in Fig. 1. The entire apparatus is then evacuated with a mechanical pump and when the best attainable vacuum has been obtained, stopcock 3 is closed thus sealing off the sample from the rest of the system. At this point the manometer is read. The thermostat is adjusted to the desired temperature and the apparatus is brought to thermal equilibrium. During this process, the mercury differential must be adjusted periodically by admitting air through capillary stopcock 1 (stopcocks 2 and 4 being open) so that the sickle gage is never subjected to an undue pressure differential. Thermal equilibrium being obtained, the sickle gage is brought to its null position in an analogous fashion and the monometer now observed. The difference between this reading and a blank (corrected for any variation in barometric pressure) represents the dissociation pressure at the temperature of the observation.

Results

Data obtained for 1,10-phenanthroline and for the 5-bromo and 5-methyl derivatives are shown graphically in Fig. 2. From a plot similar to Fig. 2, it was observed that the data could be represented with a deviation not greater than 1% by an equation of the form: $\log_{10} P = A/T + B$ in which D is an arbitrary constant and A and B are constants, the values of which depend on the units of P (pressure), T (absolute temperature), and the value of D. The thermodynamic quantities evaluated are summarized

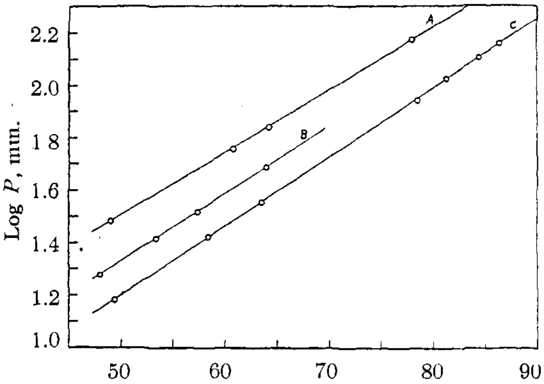

Fig. 2.

Reaction measured:

$$C_{12}H_8N_2 \cdot H_2O(s) = C_{12}H_8N_2(s) + H_2O(g)$$
$$\log_{10} P_{\text{atm.}} = -3176/T + 8.1096$$
$$\Delta F = 14500 - 37.1T \text{ cal./mole}$$
$$\Delta H = 14500 \text{ cal./mole}$$
$$\Delta S = 37.1 \text{ cal./mole/deg.}$$

Reaction measured:

$$BrC_{12}H_7N_2 \cdot H_2O(s) = BrC_{12}H_7N_2(s) + H_2O(g)$$
$$\log_{10} P_{\text{atm.}} = -2840/T + 7.3824$$
$$\Delta F = 13000 - 33.8T \text{ cal./mole}$$
$$\Delta H = 13000 \text{ cal./mole}$$
$$\Delta S = 33.8 \text{ cal./mole-deg.}$$

Reaction measured:

$$CH_3C_{12}H_7N_2 \cdot H_2O(s) = CH_3C_{12}H_7N_2\ (s) + H_2O(g)$$
$$\log_{10} P_{\text{atm.}} = -2785/T + 7.3359$$

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[9] Phipps, Spealman and Cooke, *J. Chem. Ed.*, **12**, 318 (1935).

$$\Delta F\ 13200 - 33.6T \text{ cal./mole}$$
$$\Delta H = 13200 \text{ cal./mole}$$
$$\Delta S = 33.6 \text{ cal./mole-deg.}$$

During these observations it is believed that the temperature measurements were in error by no more than 0.2° K. The sickle gage employed possessed sufficient sensitivity so that a pressure differential of 0.5 mm. could be readily detected. The mercury manometer was observed to the nearest 0.1 mm. by the use of a cathetometer.

Discussion

The reaction for which the thermodynamic quantities have been measured is the removal of a molecule of water from a molecule of 1,10-phenanthroline monohydrate, two hydrogen bonds being broken in the process. Accordingly values of 14.5, 13.0 and 13.2 cal./mole were obtained for ΔH of the hydrates of 1,10-phenanthroline, the 5-bromo and the 5-methyl derivatives, respectively. Since the nitrogen atoms in 1,10-phenanthroline are equivalent, the value of $\Delta H/2$, 7.25 cal./mole, may be considered as representing the strength of one hydrogen bond.[10] The presence of a substituent in the 5 position would be expected to affect the electron density around the two ring nitrogen atoms unequally. Therefore, it cannot be assumed that $\Delta H/2$ in these cases represents the strength of a single hydrogen bond but that it is rather the arithmetic average of the two unequal hydrogen bonds.

Compared to the O—H...O bonds measured by other workers,[5,6,7] the O—H...N bonds measured in the present work are quite strong.

It was desired to study further the effect of substituents on the strengths of the hydrogen bonds by measuring the dissociation pressures of some symmetrically 1,10-phenanthrolines. Upon searching for compounds of this class only the 5,6-dimethyl derivative was available, but contrary to expectations no hydrate could be obtained. Perhaps this is due to the extraordinarily high melting point of this compound (272° compared to 114° for the anhydrous 5-methyl compound and 117° for anhydrous 1,10-phenanthroline).

Acknowledgment.—The authors wish to thank the G. Frederick Smith Chemical Co. for the loan of the 1,10-phenanthroline chemicals, and Mr. Paul Anders who fabricated the glass apparatus employed in this investigation.

Summary

1. The dissociation pressures at various temperatures for several 1,10-phenanthroline monohydrates have been measured. From the data the thermodynamic quantities K, ΔF, ΔH and ΔS have been evaluated.

2. The value of the hydrogen bond in 1,10-phenanthroline monohydrate was found to be 7.15 cal./g. mole.

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[10] It is necessary to consider the error introduced by the possible change of the crystal energies of the solid phases due to the removal of the water molecule. According to Wenner (Wenner, "Thermochemical Calculations," McGraw-Hill Book Company, Inc., New York, N. Y., 1941, pp. 23-25) the following empirical rule is followed by organic compounds:

$$\Delta H_f/T_m = 9 \text{ to } 11$$

Where ΔH_f is the molar heat of fusion in calories and T_m the absolute temperature of the melting point. Choosing an average value of 10 for the constant we estimate ΔH_f for 1,10-phenanthroline monohydrate at 3730 cal./g. mole and for the anhydrous material at 3900 calories/g. mole. The value of ΔH_f is 200 cal./g. mole or *ca.* 100 cal./g. mole per bond. When this small correction is applied the value for each hydrogen bond becomes 7.15 cal./mole.

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY AT THE OHIO STATE UNIVERSITY]

Infrared Absorption Spectra of Some Cyclopropane and Cyclobutane Hydrocarbons

BY JOHN M. DERFER, EDWARD E. PICKETT[1] AND CECIL E. BOORD

The infrared absorption spectra of cyclopropane and cyclobutane have been determined many times and detailed theoretical analyses of these spectra have been carried out.[2]

To date the infrared spectra of only four alkyl-substituted cyclopropanes have been described in the literature. Lankelma[3] and co-workers have published the spectra of 1,1,2-trimethyl- and 1,2-dimethyl-3-ethylcyclopropane. The spectra of methyl-[4] and 1,1-dimethylcyclopropane[5] also have been published recently; a tentative assignment of frequencies was presented for the latter. A theoretical analysis of the infrared absorption spectra of 1-methylcyclobutene and methylenecyclobutane has also been carried out[5]; no spectra of other hydrocarbons of the cyclobutane series have been described.

The paucity of published spectra of substituted cyclopropanes and cyclobutanes is due chiefly to the fact that hydrocarbons of these types are relatively rare and have seldom been synthesized in quantities sufficient to allow a high degree of

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[2] (a) King, THIS JOURNAL, **58**, 1580 (1936); (b) Linnett, *J. Chem. Phys.*, **6**, 692 (1938); (c) Saksena, *Proc. Ind. Acad. Sci.*, **10A**, 449 (1939); (d) Wilson, *J. Chem. Phys.*, **11**, 369 (1943); (e) see also Bonner, *ibid.*, **5**, 704 (1937); Eyster, *ibid.*, **6**, 576 (1938).

[3] Bartleson, Burk and Lankelma, THIS JOURNAL, **68**, 2513 (1946).

[4] Condon and Smith, *ibid.*, **69**, 965 (1947).

[5] Cleveland, Murray and Gallaway, *J. Chem. Phys.*, **15**, 742 (1947).