[CONTRIBUTION FROM THE UNIVERSITY LABORATORY OF PHYSICAL CHEMISTRY RELATED TO MEDICINE AND PUBLIC HEALTH, HARVARD UNIVERSITY]

Preparation and Properties of Serum and Plasma Proteins. XXXIV. An X-Ray Study of Crystalline Human Serum Albumin Preparations[1a,1b]

BY BARBARA W. LOW

RECEIVED MARCH 13, 1952

An X-ray study has been made of two series of crystalline human serum albumin preparations. Crystals of human serum decanol albumin and of mercaptalbumin mercury dimer and its derivatives have both been examined. Measurements have been made on "wet" and on "air-dried" crystals. A value for the molecular weight of human serum mercaptalbumin of the order of 66,000 has been obtained. Limiting molecular dimensions are described.

Introduction

The preliminary X-ray study of a crystal leads to the determination of the unit cell molecular weight. The unit cell, a solid figure, contains a limited integral number of asymmetric units; usually in organic compounds these are single molecules. The number of molecules in the unit cell may generally be obtained either from the crystal symmetry, or from a knowledge of the order of magnitude of the molecular weight. In protein crystals the molecular weight of the asymmetric unit corresponds to the protein component and the liquid of crystallization associated with it. The liquid of crystallization, which is water or an aqueous solution, varies in amount in different protein crystals within a wide range, from approximately 32% in rhombohedral zinc insulin crystals to over 90% in crystals of tropomyosin.[2]

The weight fraction of water in the crystals may be determined directly by drying the crystals *in vacuo*. It may also be calculated from the crystal density by means of the formula of Adair and Adair,[3] in the special case where (1) the partial specific volume of the protein in the crystals is the same as the partial specific volume of the protein in dilute solution, and (2) the aqueous component of the crystals has a partial specific volume of 1.0.

McMeekin and Warner[4] have shown that these two conditions hold for "wet" salt free crystals of β-lactoglobulin.

From a study of the optics, size and shape of the unit cell, and general intensity distribution of the X-ray diffraction pattern, limiting dimensions for a molecule may sometimes be proposed.

The preparations examined during this study were (1) crystals of human serum decanol albumin, crystallized without ethanol from water saturated with decanol at ionic strength $\Gamma/2 < 0.001$,[5] and (2) human serum mercaptalbumin mercury dimer[6] crystallized from ethanol–water or methanol–water ($\sim 7\%$ alcohol) mixtures at ionic strength $\Gamma/2 \simeq 0.02$, and some of its derivatives.[7]

Optical and morphological measurements on human serum mercaptalbumin mercury dimer and some of its derivatives have been described.[8] The study established the isomorphism of the crystalline derivatives and crystals of the parent protein.

Experimental

The X-ray study was carried out in a cold-room with the temperature thermostatically controlled at 0°. The cold-room is entered through a vestibule in which the temperature is kept at −15°. The humidity is therefore relatively constant at all times. The "wet" crystals were mounted in thin-walled Hysil glass tubes and the diffraction patterns recorded on a Unicam plate camera. Copper Kα radiation was used.

It was noted earlier[8] that the presence of alcohol in the preparations leads to special problems in handling. The measurements were made with an over-all accuracy of approximately 1%.

Human Mercaptalbumin Mercury Dimer and its Derivatives

Molecular Weight and Hydration.—Crystals of human serum mercaptalbumin mercury dimer[9] $(Alb–S)_2Hg$ and of crystalline derivatives containing various amounts of the ions (Ba^{++}, Sr^{++} and Ag^{+}) have been examined. The crystals are orthorhombic and have the Space Group $P2_12_12$. The number of asymmetric units $n = 4$.

When the crystals are first exposed to the air a rapid change in birefringence occurs which appears to be associated with a loss of alcohol.[8] Some crystals undergo this change in the short time while they are being transferred to a glass tube for photography. The crystal in the presence of mother liquor then gives an X-ray diffraction pattern which corresponds to that of a disoriented "wet" crystal with lattice dimensions somewhat different from those of the true wet crystal. The general intensity distribution of the wet crystal is maintained. On subsequent exposure to the air these crystals are often stable and do not lose further solvent of crystallization.

The lattice dimensions, cell volume and minimum spacings observed are shown in Table I.

The mass of the "wet" crystal unit cell calculated from the density ($\rho = 1.135$)[10] and the unit cell volume is 590,000

(1) (a) This paper is Number 99 in the series "Studies on the Plasma Proteins" from blood collected by the American Red Cross, on products developed by the University Laboratory of Physical Chemistry Related to Medicine and Public Health, Harvard University. (b) This work was supported by the Eugene Higgins Trust, by grants from the Rockefeller Foundation, the National Institutes of Health, by contributions from industry, and by funds of Harvard University.

(2) M. F. Perutz, *Research*, **2**, 52 (1949).

(3) G. S. Adair and M. E. Adair, *Proc. Roy. Soc.* (*London*), **B120**, 422 (1936).

(4) T. L. McMeekin and R. C. Warner, THIS JOURNAL, **64**, 2393 (1942).

(5) E. J. Cohn, W. L. Hughes, Jr., and J. H. Weare, *ibid.*, **69**, 1753 (1947).

(6) W. L. Hughes, Jr., *ibid.*, **69**, 1836 (1947).

(7) J. Lewin, *ibid.*, **73**, 3906 (1951).

(8) B. W. Low and E. J. Weichel, *ibid.*, **73**, 3911 (1951).

(9) W. L. Hughes, Jr., *Cold Spring Harbor Symposia, Quant. Biol.*, **14**, 79 (1950). This protein is a dimer of mercaptalbumin (the fraction of serum albumin containing a single free SH-group per molecule) in which two mercaptalbumin residues are linked through their –SH groups, by a mercury atom. It can be formulated Alb.S-Hg-S.Alb, where Alb.S represents the mercaptalbumin residue.

(10) B. W. Low and F. M. Richards, unpublished studies.

TABLE I

Crystals	a, Å.	b, Å.	c, Å.	Cell volume, Å.³	d minimum, Å.
"Wet" $(Alb-S)_2Hg$	165	83	63	863,000	3
Alcohol-poor Ba^{++} and Ag^{+} derivatives	154	83	63	805,000	3.5–6.5
"Air-dried" $(Alb-S)_2$-Hg	148	50	51.5	381,000	7.5

avograms,[11] that of the "air-dried" crystal with a density (ρ = 1.29)[10] is 296,000 avograms. The "molecular weights" of the asymmetric unit are therefore 147,000 and 73,750, respectively. The weight fraction of protein in the "wet" crystal is required for the determination of the protein molecular weight. On drying *in vacuo* at 70° Richards (personal communication) found that the "dry" protein was 44.6% by weight of the "wet" crystal.

The hydration of the "wet" crystal may be calculated from the formula of Adair and Adair.[3]

$$w = \left(\frac{D_P - D}{D - D_1}\right)\frac{D_1}{D_P} \quad (1)$$

w = g. of water per g. of dry protein
D = density of crystal
D_1 = density of water
D_P = density of anhydrous protein

derived from the equations

$$\bar{v} = v_P X_P + v_{LC} X_{LC} \quad (2)$$

and

$$X_P + X_{LC} = 1 \quad (3)$$

$\bar{v}$ = specific volume of the crystal $\left(\frac{1}{D}\right)$
v_P = partial specific volume of the protein in the crystal
v_{LC} = partial specific volume of the liquid of crystallization
X_P = weight fraction of protein
X_{LC} = weight fraction of liquid of crystallization

where

w = X_{LC}/X_P
D_1 = $1/v_{LC}$ = 1.0
D_P = $1/v_P$

Using the value (0.733) reported by Oncley, Scatchard and Brown[13] for the partial specific volume of albumin, the calculated amount of protein in the "wet" crystal is 44.6%.

The unit cell contains therefore two mercaptalbumin mercury dimer molecules.[14] This gives a value for the molecular weight of mercaptalbumin mercury dimer ($(Alb-S)_2Hg$) of 131,500 and for the molecular weight of the mercaptalbumin molecule itself of 65,600 ± ~700 *i.e.* (131,500 − 200)/2.

The agreement between the measured weight fraction and that calculated from equation (1) appears to establish the validity of the assumptions made in the use of the Adair and Adair formula for "wet" protein crystals in equilibrium with their mother liquor at low salt concentration. The weight fraction of protein in the "air-dried" crystal X_P is computed from the molecular weight of the albumin derived above, and the molecular weight of the "air-dried" crystal unit cell. In the "air-dried" crystals of human mercaptalbumin mercury dimer X_P = 0.889. The partial specific volume of the albumin in the "air-dried" crystal may be calculated from equations (2) and (3) if the specific volume of the liquid of crystallization (largely water) is unity, v_{LC} = 1. This gives a value of 0.747 for the partial specific volume of albumin under these conditions.

The water in the "air-dried" crystals corresponds to 458 molecules of water per 65,600 molecular weight mercaptalbumin molecules, or 698 gram moles per 10^5 grams protein. There are 442 gram moles of polar groups (486 including proline) per 10^5 grams protein in human serum albumin.[16] Thus there is more water than is required for the hydration of the polar groups and much less than the amount "bound" in dilute solution.[17] The amount of water is probably related to the controlling effect of the molecular shape on the hydration of the molecule in "air-dried" crystals. which will be discussed below.

Molecular Size, Shape and Orientation.—The molecule of human serum albumin[13] has been described in terms of a prolate ellipsoid with major axis $2a$ = 150 Å. and minor axis $2b$ = 38 Å. (Fig. 1a). The volume of the ellipsoid corresponds to an hydrated molecule of molecular weight 69,000 and density 1/0.733 with approximately 0.2 g. of "bound" water per gram of protein.

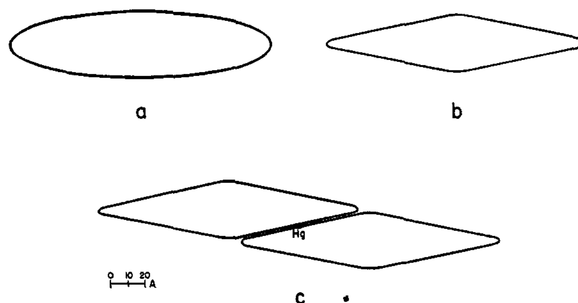

Fig. 1.—Cross section drawings of models for albumin molecule: a, prolate ellipsoid; b, right prism, rounded off at acute angle; c, mercaptalbumin mercury dimer Alb–S–Hg–S–Alb. Association of molecules at mercury link.

From viscosity and sedimentation studies on the mercaptalbumin mercury dimer Oncley[18] has suggested an alternative model of equal volume for the hydrated molecule. The molecule is described as a modified right prism approximately 150 Å. in length, 36 Å. across and 38 Å. in height. The acute edges of the prism which is shown in cross section in Fig. 1b are rounded off to approximately 3 Å. Oncley has proposed that in the dimer lateral association of two mercaptalbumin molecules of the kind shown in Fig. 1c takes place. If the sulfhydryl group occurs in identical locations on each molecule, then the –S–Hg–S bond occurs half way along the molecular overlap.

The volume occupied by an unhydrated molecule of molecular weight 65,600 and density 1/0.747 is 81,400 Å.³ ($M = V\rho N$), *i.e.*, 85% of the total

(11) The use of the term "molecular weight" to describe the weight of a volume containing an undetermined number of molecules rather than a single molecule has been criticized (J. L. Oncley, personal communication). The term avogram "the quantity of matter which is one gram divided by Avogadro's number" proposed by T. F. Young and reported by E. J. Crane[12] may be more rigorous and apt.

(12) E. J. Crane, *Chem. Eng. News*, **28**, 1842 (1950).

(13) J. L. Oncley, G. Scatchard and A. Brown, *J. Phys. Colloid Chem.*, **51**, 184 (1947).

(14) The molecular weight of albumin has been previously reported as 69,000.[13,15] This establishes the order of magnitude and characterizes the asymmetric unit as one-half of a dimer molecule.

(15) G. Scatchard, A. Batchelder and A. Brown, THIS JOURNAL, **68**, 2320 (1946).

(16) E. Brand, *Ann. N. Y. Acad. Sci.*, **47**, 187 (1946).

(17) G. S. Adair and M. E. Adair, *Proc. Roy. Soc.* (*London*), **A190**, 341 (1947).

(18) J. L. Oncley, personal communication.

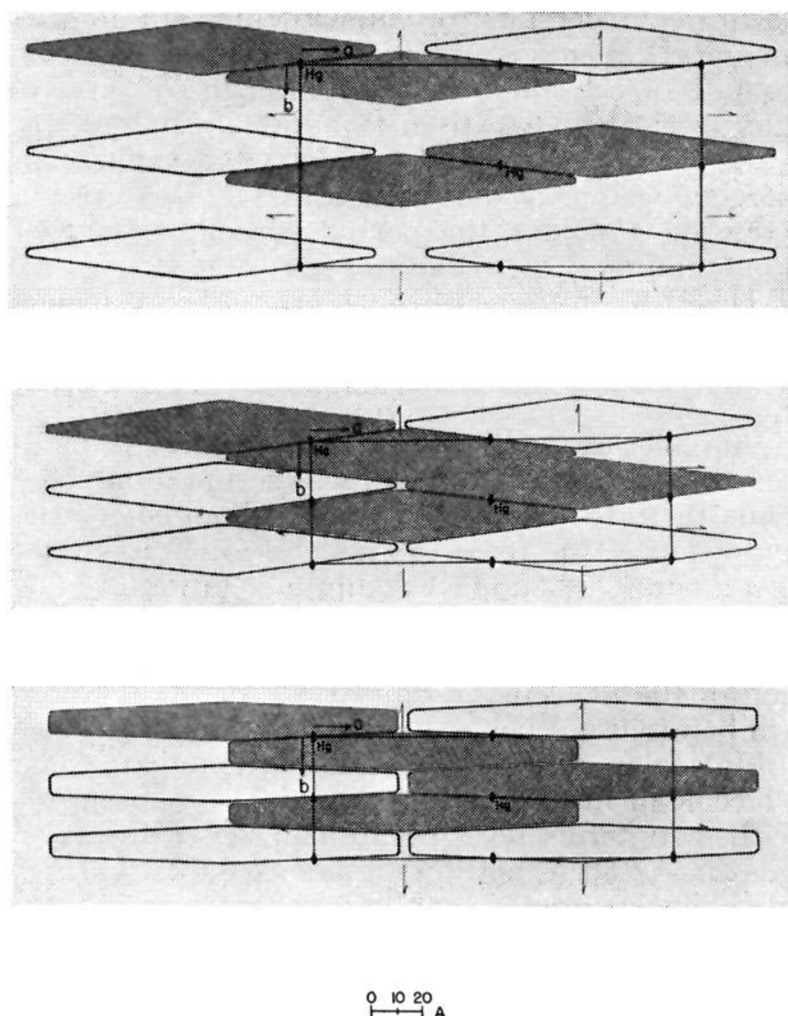

Fig. 2.—a, wet unit cell c plane projection. Packing of mercaptalbumin mercury dimer molecules. (b) and (c) "air-dried" unit cell c plane projections. Packing of two different molecular models.

volume of the asymmetric unit in the "air-dried" unit cell. The dimensions of the unhydrated molecule are consequently much smaller than those used to describe the hydrated molecule of higher molecular weight.

The morphology, cell dimensions and the intensity distribution of the X-ray diffraction pattern for mercaptalbumin mercury dimer crystals, provide evidence for the orientation and limiting shape of an elongated molecule of known volume and axial ratio approximately 4.0. The "wet" crystals of mercaptalbumin mercury dimer show perfect cleavage parallel to the (00*l*) face. The crystals split very easily and many "single crystals" are multiple lamellae sheared by slip along (00*l*).[8] The (00*l*) reflection in the "wet" crystals is very strong, the outstanding (00*l*) reflection. These observations suggest a single layer structure perpendicular to c, the direction in which the lattice shrinkage is least in measured distance.

From the unit cell dimensions, the length of the molecule must lie approximately parallel to a. Since there are only two mercury atoms in the structure these must occupy one of the two sets of twofold position ($00z$, $\frac{1}{2}\frac{1}{2}z$) or ($0\frac{1}{2}z$, $\frac{1}{2}0z$). The intensity distribution of the near-in (*hk*0) reflection is markedly face-centered. Two dimer molecules in which the orientation of the mercaptalbumin about the –S–Hg–S–bond is that proposed by Oncley fit into a pseudo-face-centered cell as shown diagrammatically in the c plane projection in Fig. 2a. In particular this orientation of the molecules leads to a very strong (020) reflection, as observed. In the "air-dried" cell the intensity distribution of the (*hk*0) reflections is not markedly face-centered, and appears to reflect the molecular asymmetry. The most prominent (0*k*0) reflection is (040), the trace of which passes through the long axes of the close-packed molecules in the "air-dried" cell. The orientation described schematically in Fig. 2 gives the index of refraction β parallel[8] to the molecular length in the dry cell.

The dimer molecules occupy too great a percentage of the total volume to be simple close-packed ellipsoids, which occupy only 74.02%[19] of the total volume of a rectangular parallelepiped.

The rectilinear models shown in Fig. 2 could be close-packed without leaving free space for water molecules. While the water in the "air-dried" crystals may be accounted for on the basis of specific hydration or non-specific capillarity of the molecular aggregate, it may also indicate the presence of some curvilinear surface. Certainly those protein crystals in which the protein molecule most closely resembles a sphere appear to contain the greatest percentage of water of crystallization in the "air-dried" crystal. The crystals of "air-dried" tobacco necrosis virus, where the water of crystallization in the "air-dried" crystals is 21.4% by weight, demonstrate this effect.[20]

The molecular shape of mercaptalbumin cannot correspond to a simple prism with sharp angles.[21] The width at the acute angle probably has the minimum value of about 3 Å. used by Oncley.[18] A maximum value of approximately 9 Å. would correspond to the diameter of a coiled peptide chain. The two modified prisms shown in Fig. 2b and c are both 144 Å. in length with a prism height of 45 Å. and are either 22 or 16 Å. wide at the center. The more blunted the edges the narrower the prism since the length of b in the models corresponds to the sum of the two edges and the two widths, *i.e.*, 50 Å. = 22 Å. × 2 Å. + 3 Å. × 2 Å. or 50 Å. = 16 Å. × 2 Å. + 9Å. × 2 Å. The mercury atom is exactly half way along the edge of the molecules in these two projections whereas in Fig. 2a it is slightly off-center. We have no criteria for discriminating between these two models. The molecular overlap must be almost half the molecular length or the packing proposed cannot be maintained. Other models with somewhat varied dimensions are equally possible on the basis of general orientation, for example 148 Å. × 22 Å. × 45 Å. or even the much shorter model 130 Å. × 22 Å. × 50 Å. Markedly non-rectilinear solids may have considerably modified dimensions.

Human Serum Decanol Albumin

Molecular Weight and Hydration.—Crystals of human serum decanol albumin prepared by Cohn, *et al.*,[5] have been examined. They form diamond-

(19) J. Boyes-Watson, E. Davidson and M. F. Perutz, *Proc. Roy. Soc.* (*London*), **A191**, 83 (1947). These authors have pointed out the volume requirements of close-packed spheres and ellipsoids. There is a slight error in the figure of 73% they give for the volume occupied.

(20) P. Cowan and D. C. Hodgkin, *Acta Cryst.*, **4**, 160 (1951).

(21) The present description of globular protein molecule is in terms of close-packed coiled polypeptide chains of circular or elliptical cross section.

shaped straw-colored plates described photographically in their paper.

The crystals, bounded by {110} are extremely soft and waxy, and show cleavage parallel to the main (001) face. Many single crystals are laminated by slip on the (001) face. The crystal shows straight extinction on the main face with β parallel to b and γ parallel to the trace of a, α is nearly (approximately 14°) parallel to c. The crystals are optically negative with a medium value for 2V. The crystals are monoclinic and have the Space Group F2 in an approximately orthogonal unit cell. The number of asymmetric units in the pseudo-orthorhombic cell is 8; in C2, the true monoclinic cell, $n = 4$. The lattice dimensions, cell volume and minimum spacings observed are shown in Table II.

TABLE II

Crystals	a, Å.	b, Å	c, Å.	β	Cell volume	d minimum, Å.
"Wet" human serum albumin	178	54	166	91°	1,596,000	3
Drying intermediate lattice	176		154	92° 40′		5
"Air-dried" human serum albumin	168	38	134	95°	856,000	8.5

The densities of the "wet" and "air-dried" monoclinic crystals of human serum albumin are 1.145 and 1.26, respectively. The mass of the "wet" cell is 1,100,000 avograms and that of the "air-dried" cell 649,000 avograms. The "molecular weights" of the asymmetric units are 137,500 and 81,100, respectively. Only one crystal was used for each density determination and these determinations are therefore probably accurate only to within 2%. The calculated amount of protein in the "wet" crystals is 47.4%, and this gives a value for the molecular weight of human serum albumin of 65,200 ± 1,300. This is in close agreement, within the limits of experimental error, with the value found for the mercaptalbumin molecule. On the basis of a molecular weight for human serum albumin of 65,600 the weight fraction of protein in the dry cell is 0.809. The water in the "air-dried" crystals corresponds to 856 molecules of water per 65,600 molecular weight albumin molecule, almost twice as much as that in the "air-dried" crystals of mercaptalbumin.

Molecular Size, Shape and Orientation.—Evidence for the molecular orientation in this crystal structure is not clear cut. The general intensity distribution observed in the "wet," "air-dried" and "intermediate" lattices does not suggest a simple molecular arrangement. The Space Group symmetry and the cell dimensions of the "air-dried" unit cell are both compatible with the packing of molecules of the general shape and size of the models discussed earlier for mercaptalbumin. The lengthwise orientation of a molecule somewhat shorter than the models in Fig. 2 and parallel to the direction of the principal refractive index α in the "wet" cell, leads to satisfactory molecular packing. The preferred length for the molecule in this structure is 138 Å.

Discussion

The X-ray study of two crystalline human serum albumin preparations has led to values for molecular weights of the albumin molecule, and of the mercaptalbumin fraction, which are of the order of 66,000. This value differs significantly from the value of 69,000 obtained hitherto.[15] Further detailed studies of these preparations in which the over-all error of measurement should be less than 1% are now proceeding. The X-ray study of a third orthorhombic crystalline preparation of human serum albumin is in progress. It should also provide limiting information concerning the molecular weight, size and shape of the albumin molecule. A molecular model based on the modified prism suggested by Oncley can pack into both crystal structures. In particular the principal features of the mercaptalbumin mercury dimer structure appear to correspond rather well to the packing of mercaptalbumin dimers in which the monomer albumin molecules are linked together, through the –S–Hg–S–bond, in the orientation proposed on the basis of viscosity and sedimentation studies. A molecular length of approximately 138 Å. is preferred since it packs equally well in both structures.

Any attempt to describe protein molecular shape in simple geometrical terms is however fundamentally inadequate. The side chains which project laterally from a coiled polypeptide chain present a highly indented surface. Certain surfaces of the molecule must be equally recessed and have the appearance somewhat of the ventral surface of a centipede. The volumes computed for protein molecules illustrate the inadequacy of the simple model. The volume occupied by a molecule of mercaptalbumin of molecular weight 65,000 and density 1/0.733 is 79,900 Å.^{3}, whereas, if the density is 1/0.747, the volume as given earlier is 81,400 Å.^{3} Similar discrepancies exist for volumes of other proteins computed in this way from the partial specific volume of the protein in "wet" and "air-dried" crystals. The studies of Neurath and Bull[22] on the volume contraction of ovalbumin during the early stages of water adsorption indicate that the partial specific volume of the protein decreases asymptotically toward the value in dilute solution. The most rapid change takes place within the range 0–30% of water. In dry proteins it is evident that packing of such complex shapes must lead to long intermolecular and side-chain van der Waals distances and therefore to a rather low density. Similarly in dry protein crystals, rather loose, though probably somewhat entangled, associations between close-packed protein molecules probably occur. It is significant that "wet" crystals, since they are the form in which protein crystals do grow from solution, must represent a potential energy minimum. "Air-dried" protein crystals give rather poor X-ray diffraction photographs which correspond to considerable intermolecular disorientation. "Air-dried" protein crystals do not return to the ordered "wet" state when the aqueous vapor pressure is increased. True reversible swelling and shrinking has only been observed within a limited range of crystalline hydration.[23] In dilute solution or in "wet" crystals the molecule is fully hydrated, the partial specific volume is reduced both by the presence of small space-filling water molecules, and also by the shortened interatomic distances (probably hydrogen bonds) between "bound" water molecules and the protein molecule. Considerations of this kind may account for the discrepancy in the volumes computed from "wet" and "dry" crystals for albumin and other proteins.

The volume of a protein molecule depends there-

(22) H. Neurath and H. B. Bull, *J. Biol. Chem.*, **115**, 519 (1936).
(23) M. F. Perutz, *Trans. Faraday Soc.*, **B42**, 187 (1946).

fore upon its physical state. The discussion is further complicated by our lack of knowledge of the partial specific volume of the lattice water. The calculations made here, of the changes in partial specific volume of the protein, depend upon the assumption that the partial specific volume of the water remains unchanged. Perutz[19] and his co-workers have adopted the alternative assumption that the partial specific volume of the protein remains constant in the crystal at all stages of drying and that the partial specific volume of the liquid of crystallization changes. Both assumptions are probably equally inadequate since the intermolecular binding between the molecules of protein and of water presumably leads to changes in the specific volume of both components of the crystals.

The formula of Adair and Adair cannot be used with accuracy for calculations on "air-dried" crystals since it is based on the assumption that values for the partial specific volume of both protein and water remain constant on drying the crystal. It does, however, appear to be applicable to the calculation of the weight fractions of protein in "wet" crystals and thus the protein molecular weights, where composition measurements are not available.

Acknowledgments.—I wish to thank Professor E. J. Cohn, who suggested this research, for his continued encouragement. I am grateful to Professor J. T. Edsall and Professor J. L. Oncley for their critical interest in this study.

BOSTON, MASS.

[CONTRIBUTION FROM DEPARTMENT OF CHEMISTRY, OREGON STATE COLLEGE]

Quinazolines. XIII. A Study of the Infrared Spectra of Certain Quinazoline Derivatives[1]

BY HARRY CULBERTSON, J. C. DECIUS AND BERT E. CHRISTENSEN

RECEIVED FEBRUARY 4, 1952

The infrared spectra of twenty quinazoline, quinazolone and quinazolinedione derivatives were obtained as a preliminary to the investigation of the structures of some anomalous quinazoline compounds. The ten compounds representing the quinazoline series are found to give rise to three bands in the "double bond" region: 1478 to 1517 cm.$^{-1}$, 1566 to 1581 cm.$^{-1}$, and 1612 to 1628 cm.$^{-1}$. These bands are designated "Quinazoline I, II and III," respectively. However, it was observed that 4-mercaptoquinazoline has an anomalous absorption in this region which indicated the possibility of a thione structure. The infrared spectra of 4-mercaptoquinazoline and 2-methyl-4-mercaptoquinazoline were obtained in the region 2500 to 3500 cm.$^{-1}$, using a lithium fluoride prism. It was found that both compounds possess a band in the N–H region but no band in the S–H region which proves the thione structure. The six compounds representing the quinazalone series were investigated and the carbonyl band was found to give rise to an absorption in the region 1637 to 1704 cm.$^{-1}$. The C=N band was more difficult to identify as conjugation and substitution effects are more pronounced. No bands which could be identified with the quinazalone ring system were observed. The quinazolinedione series was found to possess two carbonyl frequencies in agreement with other diacylimides. Two other bands found in the "double bond" region are apparently associated with the quinazolinedione ring system.

There have been few systematic investigations of ring systems to find characteristic bands which would help identify the rings. The phenyl group, of course, has been extensively investigated and three or four characteristic bands establish the presence of this group readily.[2] Randall, *et al.*,[2] have also assigned a pair of characteristic absorption bands both to the thiazole and benzthiazole rings. The lack of assigned bands for most ring systems makes it necessary to obtain a large number of spectra when undertaking a structural investigation involving some specific ring. This was the case when it was necessary in this Laboratory to use the infrared spectra as an aid in investigating the structures of certain quinazoline compounds.[3,4]

The only systematic study of absorption spectra of quinazoline derivatives published thus far is that of Elderfield, *et al.*,[5] who recorded the ultraviolet absorption spectra of quinazoline, dihydroquinazoline and related derivatives. A few isolated quinazalone compounds have had their spectra recorded in the ultraviolet but the use of ultraviolet spectra in the investigation of structures is limited by solvent effects and lack of resolution of the vibrational spectra. Since the vibrational spectrum furnishes the most information with regard to functional groups in any complex organic molecule, the decision was made to study the absorption of quinazoline compounds in the infrared. During the course of the present work the spectra of several quinazolines, quinazalones and quinazolinediones were obtained. From these data it is apparent that certain characteristic absorption bands are associated with the quinazoline ring.

Experimental

Preparation of Compounds.—The compounds used in this investigation are given in Table I. The references cited refer to the method of preparation.

Preparation of Samples for Infrared Absorption.—Two compounds of this series (4-methylquinazoline and 2,4-dimethylquinazoline) were liquids at room temperature and were observed as capillary films between rock salt windows. Samples of the low melting solids (m.p. less than 150°)

(1) The work described in this paper was made possible by grants from the Research Corporation and the General Research funds of the Graduate School of Oregon State College. Published with the approval of the Monograph Publications Committee, Oregon State College, as Research Paper No. 205, School of Science, Department of Chemistry.

(2) H. N. Randall, *et al.*, "Infrared Determination of Organic Structures," D. Van Nostrand Co., Inc., New York, N. Y., 1949.

(3) A. Tomisek and B. E. Christensen, THIS JOURNAL, **70**, 2423 (1948).

(4) C. H. Wang, T. C. Feng and B. E. Christensen, *ibid.*, **72**, 4887 (1950).

(5) R. C. Elderfield, *et al.*, *J. Org. Chem.*, **12**, 405 (1947).