Adsorption of Vapors on Organic Crystals. I. Adsorption of Water Vapor on Glycine, Leucine, Diketopiperazine and Diglycylglycine[1]

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A number of theoretical and experimental papers have appeared recently dealing with the sorption of water vapor by proteins. These have been briefly reviewed by McMeekin and Warner.[3] The adsorption isotherms obtained by Bull[4] and Shaw[5] have been discussed by Pauling[6] in terms of the amino acid composition of the protein adsorbents. He reaches the conclusion that each polar side chain in the protein is capable of binding one water molecule, this hydrated state corresponding to the adsorbed monolayer calculated from the Brunauer, Emmett, Teller[7] (B.E.T.) theory. Sorption of water by the peptide linkages is believed to be inappreciable, presumably because they are engaged in hydrogen-bonded cross linkages. The thermodynamic aspects of water sorption by proteins have been recently discussed and clarified by Dole and McLaren.[8]

The present work is an attempt to elucidate some of the problems raised by the studies on proteins, by means of an investigation of water adsorption by a series of much simpler related compounds. Three of the organic crystals chosen as adsorbents are such that the water uptake is due to surface adsorption and complications arising from sorption and solution processes are eliminated. Glycine has been chosen as the simplest example of a zwitterionic crystal; in leucine a bulky hydrocarbon side chain has been added to the structure; diketopiperazine consists of a pair of peptide bonds joined together by methylene groups. In addition there is reported an experiment on diglycylglycine, which behaves quite differently from the other crystals. It is believed that the surfaces of these crystals provide simplified models for the more complicated surfaces of proteins. One feature that may be lacking in the models is the cross linkage between peptide bonds.

With these crystals it is possible to measure the total surface area by adsorption of an inert gas, krypton being used in our experiments. This is not in general possible with proteins, the interior surfaces of which are accessible to water but not to inert gases. The water isotherms were determined at 15, 25 and 40° on crystal samples of known inert gas area.

Experimental

Apparatus.—The water adsorption apparatus was of the volumetric type similar to those which have been widely used for surface area measurements.[9] Mercury cut-offs were used throughout instead of stopcocks. Two gas burets were included, one with a volume of 475 cc. in steps of about 100 cc., while the other was a 100-cc. buret calibrated to 0.2 cc. The manometer was a closed end type, filled with Apiezon oil "B." It was read with a cathetometer to 0.005 cm., which corresponded to an accuracy of 0.004 mm. of mercury. The two burets were thermostated at 25°.

A second apparatus of the same type was used for measuring the surface areas of the crystalline samples with an inert gas, in this case krypton. This apparatus employed a McLeod gage as the pressure measuring device. The procedure followed is described in detail by Beebe, Beckwith and Honig.[10]

Materials.—The glycine was a U.S.P. product manufactured by Merck and Co. It was recrystallized from water and ground in a mortar. This mechanical process tends to develop preferentially the cleavage faces of the crystal, in this case the (010) planes. The l(−)-leucine was a C.P. product obtained from the Interchemical Corporation. It was recrystallized from water and mechanically ground to a fine particle size. The diketopiperazine, an Eastman Kodak Co. product, was recrystallized from water, air dried, and then frozen in liquid nitrogen, after which it was mechanically ground to a fine particle size. The diglycylglycine was synthesized by the choloroacetyl chloride method, and recrystallized four times from water.

The krypton used for the inert gas area measurements was obtained from the Linde Air Products Company in sealed 1-liter bulbs and introduced directly into the apparatus through a Prytz cut-off valve. The water used in the adsorption measurements was redistilled water, which was distilled in vacuo, repeatedly pumped to remove dissolved air, and then introduced from a bulb into the evacuated system.

Procedure.—Each sample, 5 to 8 g. of powdered crystals whose krypton area was previously measured,[10a] after being sealed into the system was heated in the neighborhood of 100° for twenty-four hours, while being continuously pumped by a high vacuum system, consisting of oil pump, mercury diffusion pump, and liquid nitrogen cold trap. The mercury cut-off to the sample bulb was then raised and a charge of water vapor introduced into the gas buret. The value of the charge was determined for several pressures and the sample was then exposed to the water vapor. Water vapor can be assumed to behave as an ideal gas under the conditions of these experiments.[11] The initial adsorption process was extremely slow, so that ordinarily the first points were taken at a high relative pressure and the desorption curve first obtained. The isotherms

(1) Presented in part at the 112th Meeting of American Chemical Society, New York City, September, 1947. The paper is taken from a dissertation presented by H. J. Frey in partial fulfillment of requirements for Ph.D. degree.

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(3) T. L. McMeekin and R. C. Warner, Ann. Rev. Biochem., Vol. XV, 133–139 (1946).

(4) H. B. Bull, THIS JOURNAL, 66, 1499 (1944).

(5) T. M. Shaw, J. Chem. Phys., 12, 591 (1944).

(6) L. Pauling, THIS JOURNAL, 67, 555 (1945).

(7) S. Brunauer, P. H. Emmett and E. Teller, ibid., 60, 309 (1938).

(8) M. Dole and A. D. McLaren, ibid., 69, 651 (1947).

(9) For example, L. A. Wooten and C. Brown, ibid., 65, 113 (1943).

(10) R. A. Beebe, J. B. Beckwith and J. M Honig, ibid., 67, 1554 (1945).

(10a) The krypton isotherms were well reproducible and exhibited no hysteresis on desorption, this reversibility providing a check on the freedom of the krypton from xenon.

(11) J. H. Keenan and F. G. Keyes, "Thermodynamic Properties of Steam," John Wiley & Sons, Inc., New York, N. Y., 1936.

were traversed several times for both adsorption and desorption processes, and were found to be reversible after the initial adsorption had taken place. The time required to obtain equilibrium at each point was quite long, usually about twelve hours, at 15 or 25° and at the lower relative pressures. Equilibrium was attained more rapidly at 40° and at higher relative pressures.

It was necessary in measurements of water adsorption with our apparatus to consider the uptake of water by the walls of the gas burets and connecting tubing and possibly also by the Apiezon oil in the manometer. Apiezon oil B is a pure hydrocarbon product and the solubility of water in it is probably negligible; no evidence was found of any permanent uptake of water vapor by the oil, although detailed experiments on this point were not made. A correction for background adsorption on the glass apparatus was made as follows: A charge of water vapor was introduced into the system at a pressure of 1 mm. The mercury levels in the burets were raised and the equilibrium pressure determined at each point. The observed PV product decreased with increasing pressure and the difference between the original charge and the charge at a given point was considered to be the amount adsorbed by the glass. This procedure was followed for each charge of water vapor that was used. In the subsequent adsorption runs the effective charge at each buret position was then found by subtracting the background correction from the initial charge. The correction amounted to 1 to 2% of the volume adsorbed in the 15 and 25° isotherms, and 5 to 10% int he 40° isotherms, the pressures being higher in the latter case. No correction was applied for background adsorption on the sample bulb, since its volume was small compared with that of the burets.

The correction procedure described here is not completely satisfactory, but it gave consistent charge values at different pressures and buret positions.

At the end of a water run the sample was transferred to the krypton apparatus and evacuated as before, prior to a redetermination of the krypton area.

The Adsorption Isotherms

The adsorption isotherms of water on glycine, leucine and diketopiperazine were all of the Type II in the B.E.T. classification.[12] Complete isotherms were determined at 15° over a range of relative pressures, P/P_0, from 0.05 to 0.95 for glycine and leucine, and from 0.05 to 0.70 for diketopiperazine. These are shown in Fig. 1. The volume of water vapor adsorbed per gram of adsorbent, V, is plotted against the relative pressure P/P_0. The rapid uptake of water by diketopiperazine at high relative pressures is noteworthy. At

(12) S. Brunauer, "Physical Adsorption," Princeton University Press, Princeton, N. J., 1943, p. 150.
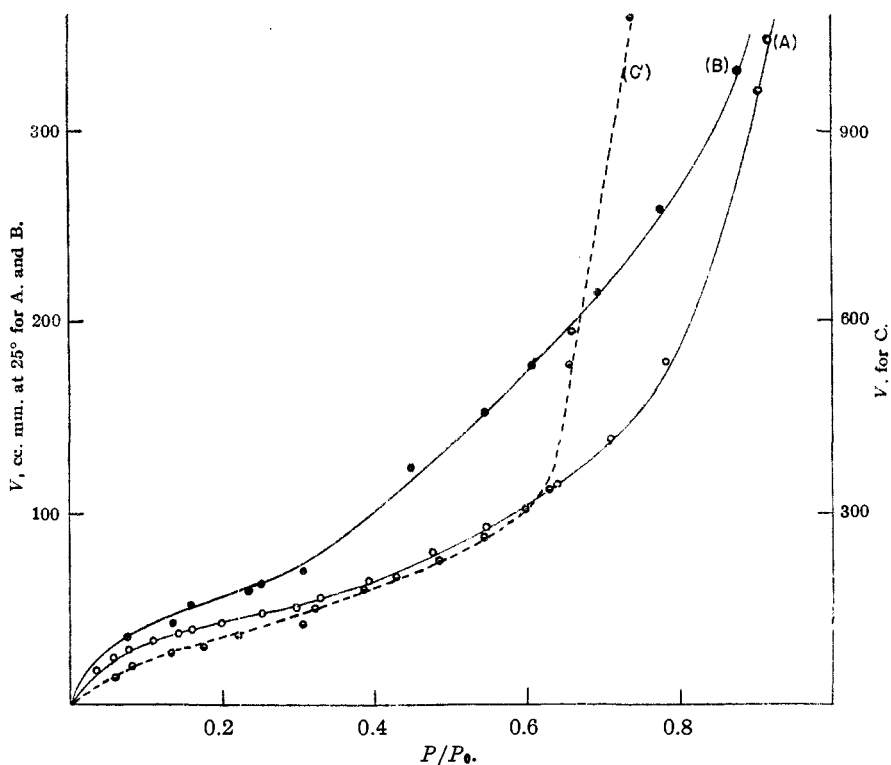

Fig. 1.—Water adsorption isotherms at 15°: (A) glycine; (B) leucine; (C) diketopiperazine.

the higher temperatures only portions of the isotherms extending to around $P/P_0 = 0.40$ were measured; in order to investigate the high pressure regions a completely thermostated apparatus would have been required. These 25 and 40° isotherms are shown in Fig. 2 and Fig. 3.

According to the B.E.T. theory these sigmoid curves indicate multimolecular adsorption. The data were plotted in terms of the B.E.T. function $P/V(P_0 - P)$ vs. P/P_0, and examples of the rectilinear plots obtained for glycine are shown in Fig. 4. Equally good rectification was obtained for the data on the other two compounds. When the data were plotted as $\log P/P_0$ vs. $1/V^2$, where V is the volume adsorbed, according to the Harkins–Jura (H. J.) relative method,[13] straight lines were not obtained from the water isotherms. The krypton adsorption isotherms on these compounds, however, yielded excellent straight lines by the B.E.T. method, and quite good straight lines by the H.J. treatment.

Comparison of Krypton and Water Areas

Surface areas of the adsorbents were calculated from the rectified B.E.T. graphs of the krypton and water isotherms. A value of 19.5 sq. Å. was used for the cross-sectional area of krypton. This value was calculated by Beebe, et al.,[10] on the basis of measurements on a sample of anatase whose area had been previously found by the

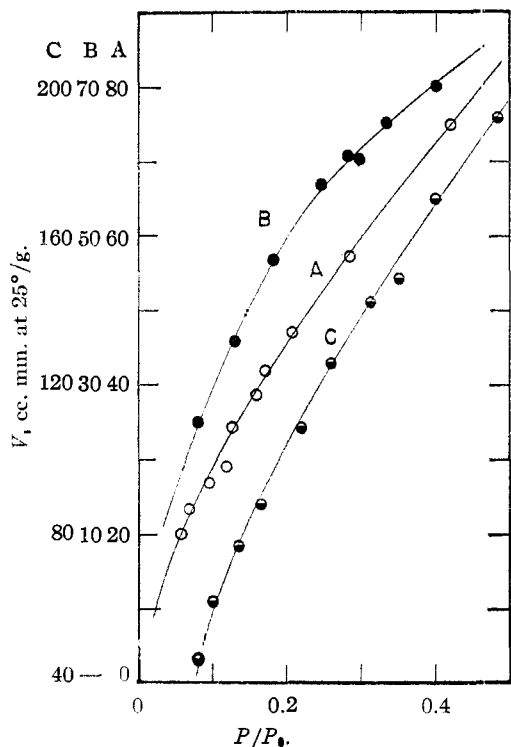(13) W. D. Harkins and G. Jura, THIS JOURNAL, 66, 1366 (1944).

Fig. 2.—25° water isotherms: (A) glycine, (B) leucine, (C) diketopiperazine.
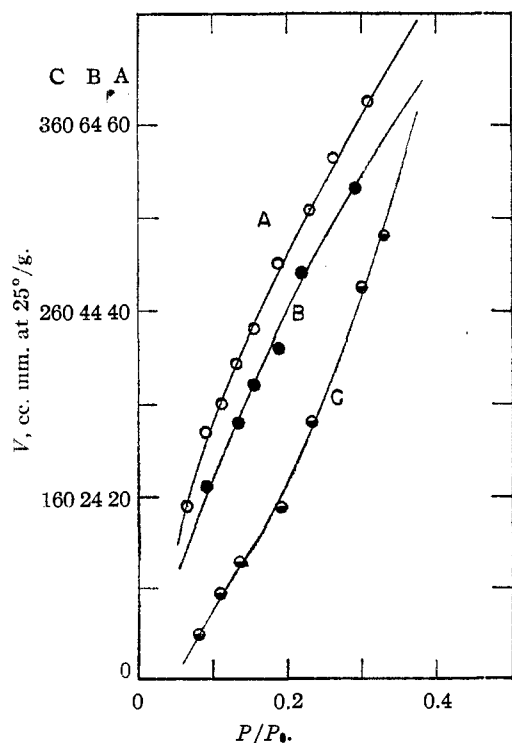

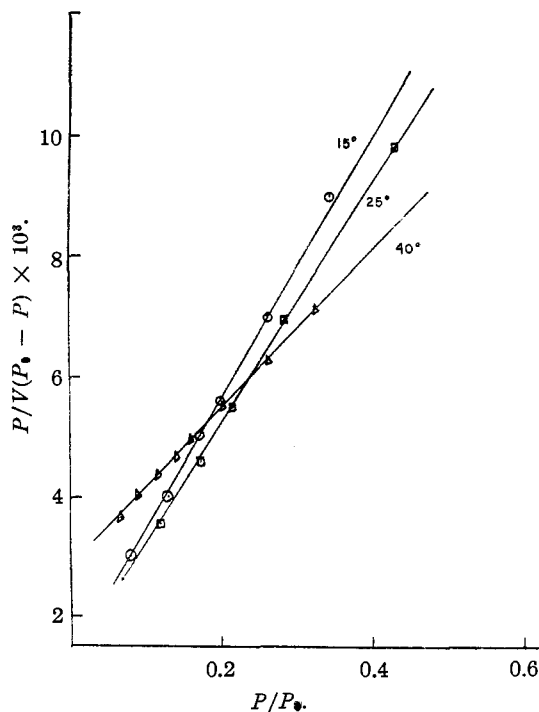Fig. 3.—40° water isotherms: (A) glycine, (B) leucine, (C) diketopiperazine.

Fig. 4.—B. E. T. plots for glycine–water.

Harkins–Jura "absolute method." The 19.5 sq. Å. value also provided the best correlation with nitrogen adsorption surface areas for anatase. For the water cross section we used a value of 10.5 sq. Å., calculated from the density of liquid water on the assumption that the adsorbed molecules pack on the surface in the same way as the molecules in the liquid in their plane of closest packing. The area chosen for adsorbed water may appear to be low, but the absolute values calculated for areas are of less interest than the differences in the area ratios for the different adsorbents. Harkins and Jura use a value of 13.8 sq. Å. for water; this value would raise the area ratios at 40° to 1.20 for glycine and 0.67 for leucine.

The krypton and water areas together with the area ratios are given in Table I, for the three compounds at the three temperatures investigated. Krypton areas were determined before and after the water runs, and there was no change in the observed values, which were reproducible to within 5%.

TABLE I

Compound	Water area,[a] 15°	25°	A_{H_2O} 40°	Krypton area,[a] A_{Kr}	Ratio, 15°	A_{H_2O}/A_{Kr} 25°	40°
Glycine	0.14	0.18	0.20	0.22	0.66	0.81	0.93
Leucine	.19	.18	.24	0.49	.38	.37	.49
Diketopip-erazine	.46	.46	.58	1.12	.41	.41	.51

[a] All areas are in square meters per gram.

Let us consider first the results for the two amino acids, glycine and leucine

+NH$_3$—CH$_2$—COO$^-$
Glycine
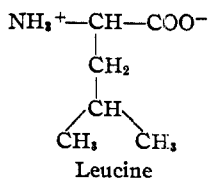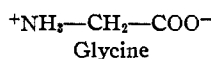
NH$_3$$^+$—CH—COO$^-$
      |
      CH$_2$
      |
      CH
    CH$_3$ CH$_3$
Leucine

The area ratios of 0.66 to 0.93 for glycine and 0.38 to 0.49 for leucine suggest that adsorbed krypton covers the entire surface of the crystal, while adsorbed water covers only the zwitterionic NH$_3$$^+$ and COO$^-$ groups. The crystal structure of glycine has been determined by Albrecht and Corey.[14] The crystals are monoclinic, with a_0 = 5.10 Å., b_0 = 11.96 Å., c_0 = 5.45 Å., β = 111° 38'. The molecules are linked by hydrogen bonds into double layers arrayed normal to the b axis. Cleavage occurs between these layers, the cleavage planes being the (010). If it is assumed that this face is practically exclusively developed in our powdered samples, it can be readily calculated, using conventional atomic radii, that 65% of the total surface area of glycine should be covered by polar groups. This figure agrees very well with the observed water to krypton ratio at 15°. Inspection of the (010) face of the glycine unit cell indicates that there are two polar groups (1 NH$_3$$^+$ and 1 COO$^-$) in each unit (010) face. Since the area of the unit (010) face is 25.8 sq. Å., there are 7.75 × 10^{14} polar groups per sq. cm. of crystal surface. If one assumes that the B.E.T. monolayer point for water occurs when each polar group binds one water molecule, the volume of water adsorbed per monolayer can be calculated from this figure and the krypton area of the sample. This calculated V_m is 42.1 cc. (0° and 1 mm. pressure) per gram of glycine. The V_m's calculated from the water isotherms are 38.3 cc. at 15°, 47.0 at 25°, and 54.4 cc. at 40°. This correlation lends support to the idea that only the polar area adsorbs water at low pressures.

The crystal structure of leucine is not known and therefore an accurate calculation of the percentage of polar area cannot be made. By projection of a molecular model, however, an estimate of 40% polar surface may be made. This is in general agreement with the somewhat higher observed ratios.

The water/krypton area ratios for diketopiperazine again suggest that only a portion of the surface is covered with water at the B.E.T. monolayer point. The crystal structure of diketopiperazine has been analyzed by Corey.[15] Diketopiperazine has the structural formula

       CO
    HN   CH$_2$
    H$_2$C   NH
       CO

The crystals are monoclinic, with a_0 = 5.19, b_0 = 11.50, c_0 = 3.96 Å., β = 83 = 1°. Cleavage occurs along (010) and (101) planes. The (101) planes consist of flat diketopiperazine rings linked into chains by hydrogen bonds. A calculation similar to that for glycine shows that these cleavage planes contain 5.7 × 10^{14} carbonyl groups per sq. cm. If one water molecule is bound to each carbonyl at the monolayer point, the V_m should be 181 cc. at 0° and 1 mm. For the (010) planes the situation is less straightforward, since one C–O group is practically in the cleavage plane while another is buried somewhat deeper. This plane can be considered as cutting the chains lengthwise. If only the one C=O group is accessible the calculated V_m would be 155 cc. The observed V_m's for water on diketopiperazine are 124 at 15°, 124 at 25°, and 151 at 40°. It may therefore be suggested that the water adsorption on diketopiperazine can be fairly adequately explained by the uptake of one water molecule per carbonyl group. The difference between the calculated and the observed V_m's is perhaps due to uncertainty in the krypton cross section or to unavailability of some of the C=O groups due to hydrogen bonding in the crystal itself.

Sorption by Diglycylglycine.—Only one isotherm, at 15°, has been completed with diglycylglycine, but in view of its interest and applicability to the problem of water sorption by proteins, the result will be briefly mentioned. Once again S-shaped isotherms were obtained with both water vapor and krypton, which yielded linear B.E.T. plots. It was now found, however, that the apparent surface area from the water isotherm, instead of being some fraction of the krypton area, was in fact six times that area. The isotherms are shown in Fig. 5 and the large water adsorption is very evident. It would appear that even with an organic crystal as simple as diglycylglycine one is beginning to approach a situation similar to that in the protein–water systems, where the sorption is not purely a surface phenomenon. It should be possible therefore to investigate some of the problems of "swelling" and water sorption with organic crystals of known structure.

Application of the B. E. T. Theory.—Recent statistical treatments of the adsorption isotherm[16] have shown that the B.E.T. type of theory is based on an adsorption on localized sites, rather than the formation of a mobile adsorbed monolayer. It is not surprising, therefore, that the water isotherms on amino acids should follow the B.E.T. equation. Water tends to be strongly adsorbed on the active polar sites, the completion of the first monolayer probably corresponding to the coverage of these sites. The hydrocarbon-like portion of the surface offers less attraction to water than does the water already attached to the active sites. Additional water will

(14) G. Albrecht and R. B. Corey, THIS JOURNAL, **61**, 1087 (1939).

(15) R. B. Corey, *ibid.*, **60**, 1598 (1938).

(16) T. L. Hill, *J. Chem. Phys.*, **14**, 263 (1946); A. B. D. Cassie, *Trans. Faraday Soc.*, **43**, 615 (1947).
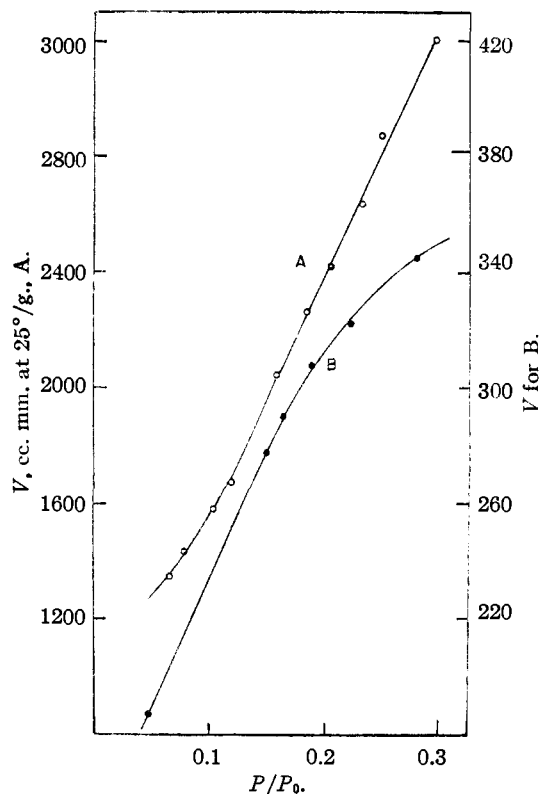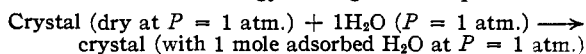
Fig. 5.—Krypton and water isotherms on diglycylglycine:
(A) water at 15°, (B) krypton at −195°.

therefore probably be adsorbed around the water
on the active sites, so that clusters of water form
around these points. These may grow both hori-
zontally and vertically, and eventually should run
together. This model is consistent with the ob-
served conformity to the B.E.T. isotherm and
lack of conformity with the Harkins–Jura con-
densed film isotherm.

Thermodynamic Data.—One may define a
standard integral free energy of adsorption per
mole as the free energy change in the process

Crystal (dry at $P = 1$ atm.) $+$ $1H_2O$ ($P = 1$ atm.) $\longrightarrow$
crystal (with 1 mole adsorbed H_2O at $P = 1$ atm.)

The free energy change in this process is given by

$$\Delta F^0 = - \int_0^x \frac{V}{x} dx + RT \ln x + RT \ln P_0/760$$

Here V is the volume of water adsorbed in cc. at
S.T.P., $x = P/P_0$ is the relative pressure of the ad-
sorbate, and P_0 is the vapor pressure in millimeters
of mercury. The integrals $\int_0^x (V/x)dx$ have been
calculated from the 15° isotherms by the method
of Boyd and Livingston.[17] Plots of the integral
free energies of adsorption are included in Fig. 6.

From the relation $\Delta \bar{F}^0 = RT \ln P/760$ it is pos-
sible to obtain values for the differential free en-
ergy of adsorption, $\Delta \bar{F}^0$, i. e., the standard free

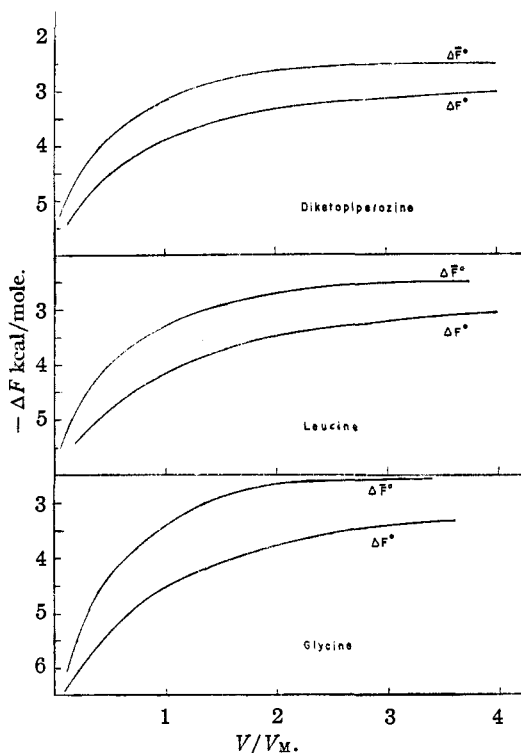(17) G. E. Boyd and H. K. Livingston, THIS JOURNAL, **64**, 2383
(1942).

energy change in adsorbing one mole of water on
the crystal surface at constant coverage, V/V_m.
These values are included in Fig. 6. By the appli-
cation of the Clapeyron–Clausius equation to the
adsorption isosteres, a series of "differential"
enthalpies of adsorption can be determined. A
typical plot of log P vs. $1/T$ is shown in Fig. 7 for
the case of glycine; the $\Delta \bar{H}$ values are obtained

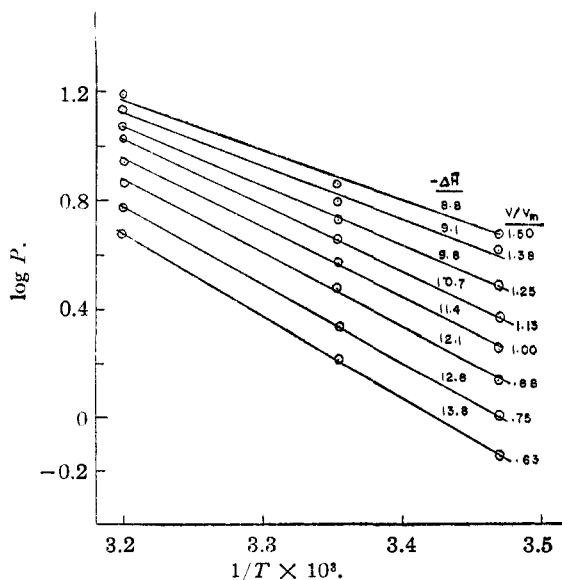Fig. 6.—Differential and integral free energies of adsorp-
tion.

Fig. 7.—Isosteric heats of adsorption.

from the slopes. If we assume that the water vapor behaves as an ideal gas, these $\Delta\bar{H}$'s can be taken as the standard differential enthalpy changes, $\Delta\bar{H}^0$. These differential enthalpies are combined with the differential free energies to yield values for the entropy of adsorption. In Table II the thermodynamic results are summarized for the three compounds at various surface coverages. These values may be compared with

TABLE II

THERMODYNAMIC DATA FOR WATER ADSORPTION AT DIFFERENT COVERAGES

	$\Delta\bar{H}^0$, kcal./mole	$\Delta\bar{F}^0$, kcal./mole	$\Delta\bar{S}^0$, cal./degree
$v/v_m = 0.5$			
Glycine	−14.40	−4.19	−35.5
Leucine	−15.25	−3.96	−39.2
Diketopiperazine	−15.20	−3.68	−40.2
$v/v_m = 0.75$			
Glycine	−12.75	−3.73	−31.4
Leucine	−13.42	−3.52	−34.4
Diketopiperazine	−12.45	−3.32	−31.7
$v/v_m = 1.00$			
Glycine	−11.10	−3.40	−26.7
Leucine	−11.70	−3.14	−29.6
Diketopiperazine	−11.08	−3.03	−28.0

those for the standard enthalpy, free energy, and entropy of condensation of water vapor to liquid water at 15°: $\Delta F^0_{288} = -2340$; $\Delta H^0_{288} = -10,-610$; $\Delta S^0_{288} = -28.72$. It is evident that the driving force for the condensation of water on the polar groups of these crystals at $V/V_m = 1$ arises mainly from the lowered energy of the sorbed films as compared with that of liquid water. The affinity of water for the ionic ends of the zwitterions is only slightly greater than its affinity for the peptide linkage in diketopiperazine, in which the carbonyl group is probably the active portion. The entropy of adsorption at the monolayer point is not notably different from the entropy of condensation of water vapor. At lower coverages the entropy decreases are more marked, suggesting a decreased mobility of the adsorbed water molecules. This "localization" is especially evident for water on leucine, where the polar regions of the surface are separated by large hydrocarbon regions. This decreased entropy is compensated by an increased heat of adsorption at lower coverages.

Sorption of Water Vapor by Proteins.—In the systems described in this paper the adsorbents were crystalline and the adsorption is believed to be essentially a surface phenomenon. The sorption of water vapor by proteins, on the other hand, is believed to occur in the interior portions of the structure, which are not accessible to inert

gases such as krypton. For example, krypton adsorption measurements on a sample of silk fibroin very similar to that used by Bull[4] yielded a surface area of 0.46 sq. m./g., compared with Bull's value of 144 sq. m./g. from water sorption. The present work indicates that a crystal like diglycylglycine sorbs water to an intermediate extent. It is a transition case between the protein-like sorption and the purely surface adsorption of diketopiperazine, glycine and leucine. It is evident from the behavior of diglycylglycine and from the thermodynamic data for diketopiperazine that the peptide link has a strong affinity for water. Recent measurements by Mellon, Korn and Hoover[18] have shown that tetraglycine, hexaglycine and polyglycines absorb large amounts of water vapor, and this sorption can be ascribed to the peptide linkages in these compounds.

The demonstrated affinity of the peptide linkage for water may lead to a reconsideration of Pauling's analysis of Bull's data.[6] Accessible peptide linkages probably contribute to the sorption even before the monolayer point, but the quantitative correlation of water sorption with numbers of polar side chains indicates that this contribution may be small at low relative pressures, increasing as the protein swells and further peptide links become accessible. Further quantitative data on the protein–water isotherms at low relative pressures may serve to elucidate these problems.

Summary

Adsorption of water vapor on powdered crystalline samples of glycine, leucine and diketopiperazine, of known inert gas surface area, was investigated at 15, 25 and 40°. The water adsorption appeared to occur primarily on the ionic COO^- and NH_3^+ groups of the amino acids and on the peptide linkages in diketopiperazine. Thermodynamic treatment of the adsorption isotherms indicates that the free energy of adsorption at the monolayer point is about 1000 calories less than the free energy of condensation of water vapor to liquid water. Diglycylglycine sorbed water to a much greater extent than the other crystals and appears to provide an intermediate case between surface adsorption and the extensive sorption observed with proteins.

(18) E. F. Mellon, A. H. Korn and S. R. Hoover, "Abstracts," 113th meeting American Chemical Society, April, 1948, p. 31F.