A STUDY OF SOME OF THE CHEMICAL CHARACTERISTICS AND THE ABSORPTION SPECTRUM OF CYSTINE.

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The present study was undertaken primarily to corroborate the work of Ward (1), who suggested from data obtained during the study of the absorption spectra of some biochemical products that cystine might possess a ring structure rather than an aliphatic structure. In the course of the preparation of cystine for this investigation some interesting results on the relation between the concentration of the cystine and the pH value at which it crystallizes or precipitates were obtained.

Cystine was prepared from wool by acid hydrolysis according to the method of Folin (2) with certain modifications suggested by Gortner and Sinclair (3). The best yield of crude cystine was obtained at pH 4 (4). This crude cystine was then dissolved in the least possible amount of 3 per cent hydrochloric acid, perfectly decolorized, and pure cystine in the familiar hexagonal, plate crystals was obtained. At a pH value of 4 to 5 some of the same crude cystine gave abundant powdery spherical bodies, which under the high power microscope resolved themselves into thin, blunt prisms. These were probably the isomeric form obtained by Gortner on boiling the crude cystine for a prolonged time in hydrochloric acid. It is known that cystine can be precipitated by the use of sodium acetate from solutions of varying hydrogen ion concentrations (4), a fact which accounts for the differences noted in the literature on cystine. A precipitation of a white powder, which resolved itself into very fine needles, was always obtained at a pH value of 5 to 6.

It was found that there was a direct ratio between the concentration of the cystine and the pH value at which it crystallized, or was precipitated. Different quantities of purified cystine were dissolved in 20 cc. of 3 per cent hydrochloric acid, 0.1, 0.2, 0.3, and 0.4 gm. To each were added 5 cc. of concentrated solution of sodium acetate. Only the most concentrated solution precipitated. Upon addition of 5 cc. to the remaining, unprecipitated solutions, the one containing 0.3 gm. of cystine precipitated.

TABLE I.

pH Values at Which Different Concentrations of Cystine Precipitate.

Sample No.	Cystine.	Concentration of HCl.	pH value.
	gm.	*per cent*	
I	0.4	3	Strong acid.
II	0.3	3	4
III	0.2	3	5.2
IV	0.1	3	6.5

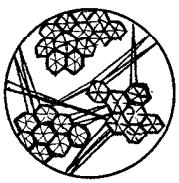


FIG. 1.

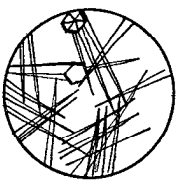


FIG. 2.

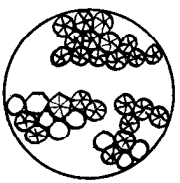


FIG. 3.

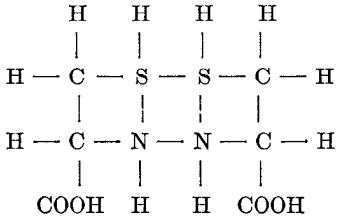
FIG. 1. Character of cystine crystals obtained in strong acid.
FIG. 2. Character of cystine crystals obtained at pH = 3.
FIG. 3. Character of cystine crystals obtained at pH = 5.

Further additions of sodium acetate were needed to bring about precipitation in the other solutions, as shown in Table I.

The character of the crystals obtained from solutions of different pH values is illustrated in Figs. 1 to 3.

Ward's proposal (1) that the structure of cystine is not that usually assumed, that of the aliphatic group, but a ring structure, was based on the fact that the magnitude of the molecular absorption coefficients of cystine in the region of the ultra-violet spectrum studied were nearly as large as those found for such

substances as tyrosine, tryptophane, and phenylalanine, which are known to have ring structure, and much larger than for alanine and glutaminic acid, which have chain structure. On this basis he proposed the following formula for the structure of cystine.



An examination of his curves, however, shows that each of the group of substances which has ring structure, produces selective absorption, a phenomenon characteristic of other substances with that structure, as has been found, for example, by van Gulick (5) for chlorophyllan, by Henri (6) for anthracene, and by Campbell (7) for uric acid. Amino acids without a ring structure do not possess the property of selective absorption but produce only continuous absorption.

The authors have carried out a similar analysis with the two forms of cystine, which were prepared in our laboratory, using the plate form, *l*-cystine, dissolved in hydrochloric acid and the needle form, *i*-cystine, dissolved in water. Different concentrations were used, and in the case of the acid solution the ratio of the molecular concentration of cystine and the percentage of hydrochloric acid was kept constant.

We used a rotating sector photometer with a small quartz spectrograph, furnished by the Gaertner Scientific Corporation. The source of light was a hydrogen discharge tube, constructed for us at the United States Bureau of Standards, which gave a continuous spectrum (8) far into the ultra-violet when run by a 1 kilowatt transformer at a pressure of several mm. Because of the homogeneity and intensity of this source it is preferred to the many lined spectrum produced in the under water spark commonly used. A Baly tube with quartz end-plates was filled with the solution and placed between the source and the lower sector of fixed aperture, and a similar tube filled with the solvent was placed between the source and the upper sector of variable aperture.

The molecular absorption coefficient represents the percentage of the light absorbed per cm. of path by a liquid of unit molecular concentration. It was calculated in the usual way from the formula

$$K = \frac{\alpha}{d\,C}$$

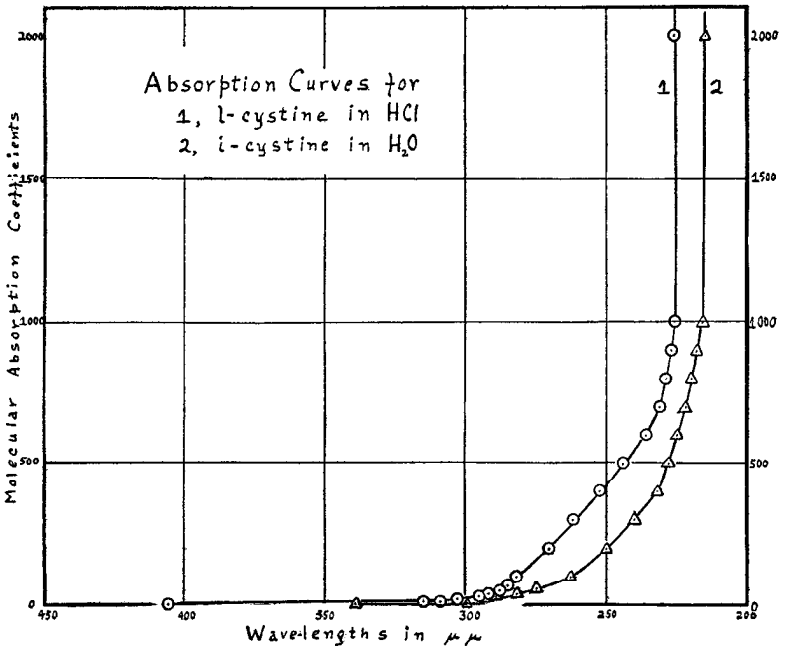


FIG. 4.

where K is the molecular absorption coefficient, α the extinction coefficient read directly on the adjustable sector of the photometer, and C the molecular concentration.

Our results are shown in Fig. 4 where we have plotted the molecular absorption coefficient against the wave-length of the light. The curves obtained when the plate form of cystine was dissolved in different percentages of hydrochloric acid were practically identical, and agree with that obtained by Ward within

experimental error. His values were plotted on a logarithmic scale because of their great range which led to the apparent irregularity near 300 $\mu\mu$. As the diagrams show, the curves are perfectly smooth when the values are plotted on a linear scale. Fig. 5 shows the lower part of our curves in greater detail. The curve obtained with needle cystine in water is also perfectly smooth, but lies further in the ultra-violet.

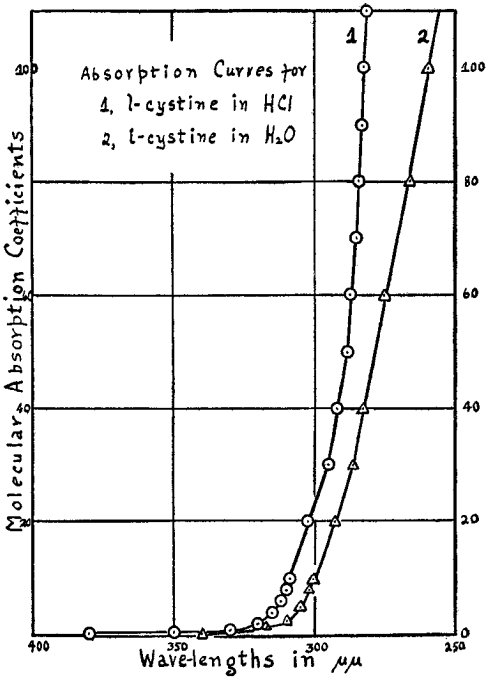


FIG. 5.

We repeated the experiment with alanine, and the results are plotted in Fig. 6. It is of the same type as those obtained with cystine, but begins further in the ultra-violet. Because of the limits of sensitivity of the plates and the length of the path of light it was difficult to carry the measurements further, but the molecular absorption coefficients undoubtedly increase in value as the wave-length of the light absorbed decreases.

The absorption curve of tyrosine, obtained by Ward, has been reproduced in Fig. 7 for purposes of comparison, a linear scale being used in plotting. This shows the character of the selective absorption obtained with substances of ring structure. If cystine has the ring structure suggested by Ward its absorption curve should be similar to this curve, or possibly, like anthracene, should have two regions with selective absorption. If, on the other hand,

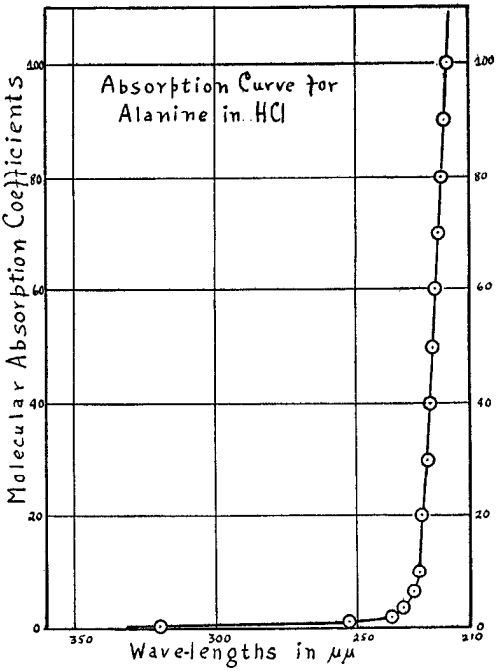


FIG. 6.

it has the chain structure, the absorption curve should be like those of alanine and glutaminic acid (1), which show only continuous absorption. Since the curve is perfectly smooth we conclude that the hypothesis of a ring structure for cystine is untenable.

The continuous absorption shown in the spectrum of cystine is attributed to the dissociation of the molecule. The absorption begins at about 400 $\mu\mu$ in the case of the acid solution and the intensity of the absorption increases as the wave-length of the

light absorbed decreases; *i.e.*, as the energy of the quantum identified with the light increases. The degree of dissociation may be calculated by dividing the mass absorption coefficient by the number of molecules per cm. of path in unit molecular concentration. The value of this coefficient at 400 $\mu\mu$ is approximately 2×10^{-8}, which is of the same order of magnitude as the value obtained by Sano (9) for the dissociation constant of an acid

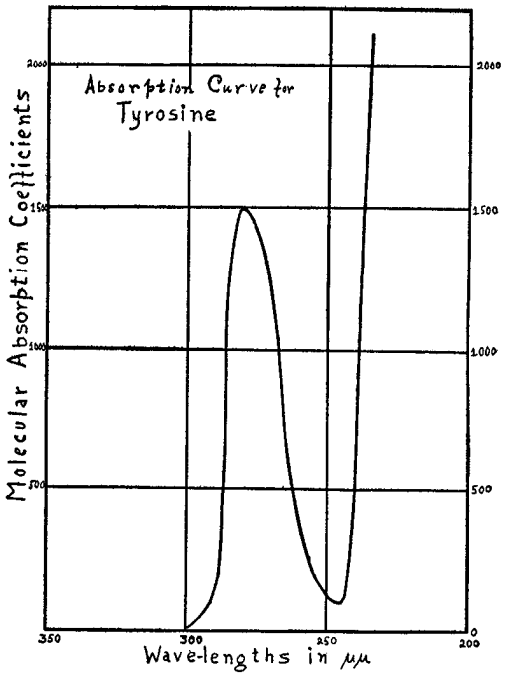


FIG. 7.

solution of cystine under normal conditions. The degree of dissociation produced by light of long wave-length remains small, 10^{-8} to 10^{-7}, probably because there are all possible values for the angle between the direction of the momentum of the light quantum and the axis of the molecule, so that in general the magnitude of the component of the momentum in the direction necessary to produce dissociation is insufficient. But as the energy of the quantum increases the probability that this compo-

nent will be sufficient increases, with the result that the degree of dissociation at 226 $\mu\mu$ is over 10^{-4}.

The energy required to produce dissociation may be calculated from the equation, $E = \frac{hc}{\lambda}$, where h is Planck's constant, c is the velocity of light, and λ its wave-length in cm. Thus at 400 $\mu\mu$, the wave-length where absorption begins, the energy required to dissociate cystine is calculated to be 4.9×10^{-12} erg per molecule, which corresponds to an ionization potential of 3.7 volts.

The heat of dissociation per mol is accordingly,

$$\frac{E \times \text{No. of molecules per mol}}{J} = 70{,}800 \text{ calories.}$$

The absorption curve shows that dissociation is complete at 226 $\mu\mu$, which represents an energy of dissociation of 8.7 erg per molecule, or 124,000 calories per mol.

For the cystine dissolved in water, dissociation starts at approximately 340 $\mu\mu$, and is complete at 215 $\mu\mu$, which indicates that slightly more energy is required to dissociate this solution than is required in the case of the acid solution.

Similarly, the energy necessary to produce the dissociation of alanine is larger than that for cystine since the absorption curve lies further in the ultra-violet. This is probably due to the fact that it is less difficult to dissociate cystine since it is a heavier molecule, and since the molecular bond is probably broken between the sulfur atoms, which we know are extremely active.

SUMMARY.

There appears to be a direct ratio between the concentration of the cystine and the pH value at which it crystallizes, or is precipitated.

Since the absorption curves for cystine show only continuous absorption we conclude that the structure of cystine is that usually assumed, a straight chain, and not a ring as proposed by Ward.

From the magnitude of the molecular absorption coefficient we have determined the degree of dissociation, produced by light of different wave-lengths, which ranges from 10^{-8} to 10^{-4}, the former value agreeing with that of Sano.

We have also calculated the dissociation energy which is ap-

proximately 4.9 erg per molecule, or 70,800 calories per gram molecule.

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