2 - Ethylmercapto - 4 - phenyl - 6 - thionethylurethanpyrimidine.—By digesting the isothiocyanate with ethyl alcohol for a few minutes, this thionethylurethan was formed smoothly. After recrystallization from benzene–petroleum ether, it separated in colorless needles, melting at 115–116°. *Anal.* Calcd. for $C_{15}H_{17}ON_3S_2$: N, 13.16. Found: N, 13.2, 13.1.

2 - Ethylmercapto - 4 - phenyl - 6 - thion - *n* - propylurethanpyrimidine.—This recrystallized from ethyl alcohol and melted at 97–98°. *Anal.* Calcd. for $C_{16}H_{19}ON_3S_2$: N, 12.61. Found: N, 12.6, 12.7.

2 - Ethylmercapto - 4 - phenyl - 6 - thion - *n* - butylurethanpyrimidine.—Prepared by action of butyl alcohol on the isothiocyanate. After recrystallization from ethyl alcohol or methyl alcohol, it melted at 89–90°. *Anal.* Calcd. for $C_{17}H_{21}ON_3S_2$: N, 12.10. Found: N, 12.1, 12.2.

Experimental Conditions Influencing the Rearrangement.—Heating the normal thiocyanate in benzene, toluene and xylene at their respective boiling points did not bring about any change. The thiocyanate could be distilled at 204° at 1.5 mm. pressure without decomposition and without conversion into the isothiocyanate. The rearrangement was, however, brought about by heating the thiocyanate at 160–170° in toluene under pressure.

Summary

1. 2-Ethylmercapto-4-phenyl-6-thiocyanopyrimidine, prepared from the corresponding chloro compound, was identified as the normal thiocyanate by its characteristic reaction with thioacetic acid.

2. Heating 2-ethylmercapto-4-phenyl-6-thiocyano-pyrimidine at 160–170° in toluene brought about a rearrangement to 2-ethylmercapto-4-phenyl-6-isothiocyano-pyrimidine.

3. The thiocyanate was non-reactive toward ammonia, aniline and alcohols. The isothiocyanate reacted with ammonia, aniline and alcohols, giving the corresponding thioureas and thiourethans, respectively.

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[CONTRIBUTION NO. 551 FROM THE KODAK RESEARCH LABORATORIES]

The Estimation of the Total Basic Amino Acids in Gelatin by Titration in Glacial Acetic Acid[1]

BY J. RUSSELL AND A. E. CAMERON

In the titration of gelatin in aqueous solution, no very significant data are obtainable with respect to groups with a *pK* above 12, due to the uncertainties introduced by the corrections for free base. Since arginine with a *pK* of 13 is found among the hydrolytic products of gelatin, it was very desirable that a method be found of supplementing the information obtained by aqueous titration. Acetic acid suggested itself as a very suitable solvent for further work, since it is a strongly acid solvent and the work of Conant[2] has shown that in it guanidine acts as a titratable strong base.

It was at first found impossible to proceed, since gelatin was insoluble in glacial acetic acid. However, the expedient was adopted of first dissolving the gelatin in a small amount of water and diluting with glacial acetic acid. The titrations were thus finally carried out in acetic acid with about 2% water present.

Portions of the solutions, containing 0.2 g. of dry gelatin, were pipetted into the titration vessel. Four palladium black electrodes were used. A half cell of chloranil–tetrachlorohydroquinone mixture in molar sulfuric acid in glacial acetic acid with a salt bridge of a saturated acetic acid solution of lithium chloride completed the cell. Hydrogen, saturated with acetic acid, was passed in at the bottom of the vessel through a porous bubble head. The exit gas passed out through a bubble bottle so that a slight positive pressure of hydrogen was maintained in the titration vessel at all times. The paraffined corks carrying the electrodes and salt bridge were sealed into the flask necks with paraffin. A swirling motion was given the flask by an electric motor to stir the solution and give the most satisfactory readings.

Freshly plated palladium black electrodes were employed in each titration. Attainment of the initial equilibrium was slow. Therefore, whenever possible, the solutions were allowed to remain overnight with hydrogen bubbling through, before beginning the titration. Four electrodes agreed uniformly within one millivolt even after precipitation of gelatin was taking place. The only effect of the precipitated gelatin was to retard the attainment of electrode equilibrium.

An approximately 0.01 *M* perchloric acid solution was prepared by adding 1 ml. of 70% aqueous acid to a liter of glacial acetic acid. Standard sodium acetate solution was prepared by dissolving a weighed amount of dried sodium carbonate in acetic acid and making up to volume. Samples of this solution were pipetted out and titrated with the perchloric acid for standardization.

The gelatin solutions employed were made up using 5 ml. of water to melt them in each case, and making up to 250 ml. total volume with glacial acetic acid.

The gelatin, measurements for which are reported in this

(1) Presented before the Leather and Gelatin Division at the New York meeting of the American Chemical Society, April, 1935.

(2) J. B. Conant and N. F. Hall, THIS JOURNAL, 49, 3047 (1927).

paper, was Eastman Purified Gelatin, Lot No. 63, which was prepared by the usual de-ashing procedure[3] from a lime-treated calfskin. A solution was made of 1.103 g. of

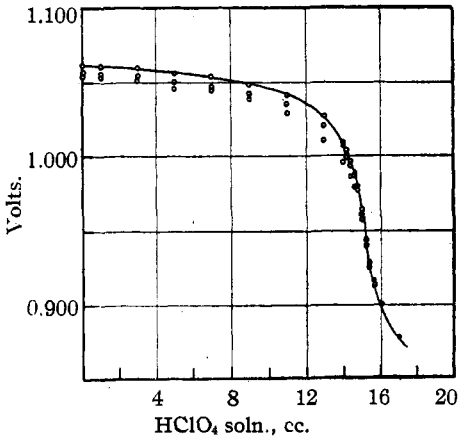

Fig. 1.

gelatin, equal to 0.938 g. of gelatin corrected to dry basis (15% moisture), in a volume of 250 cc. For 50 cc. of this solution there was required for neutralization 15.1 cc. of perchloric acid solution (0.01190 *N*). This gives a value of 9.6×10^{-4} equivalent of basic material present in one gram of dry gelatin. The titration curve is shown in Fig. 1.

For purposes of comparison, samples of two diamino acids were made up in approximately 0.02 *M* solutions and titrated with the same perchloric acid.

Lysine.—A solution was made of 0.112 g. of lysine dihydrochloride dissolved in water and treated with washed silver carbonate. The precipitate was filtered, washed, and the filtrate evaporated nearly to dryness, 20 ml. of acetic acid was added, and evaporation again carried almost to completion. The remaining solution was made up to 100 ml. with glacial acetic acid.

Arginine Monohydrochloride.—A solution was made of 0.2085 g. of the arginine monohydrochloride dissolved in 100 ml. of acetic acid solution. The salt was sparingly soluble and heating was necessary. Upon cooling, the salt did not separate out.

Arginine.—A solution was made of 0.117 g. of arginine carbonate dissolved in warm acetic acid and made up to 100 ml. total volume.

The solutions of the free bases, lysine and arginine, exhibited one peculiarity, namely, that the reliable readings of the palladium black electrodes appeared to be obtained by stopping, shaking and turning off the hydrogen to avoid any stirring.

Shaking caused the potentials to drop and readings of individual electrodes to diverge.

The curves for the titration of the amino acids are shown in Fig. 2. The end-points indicate that both basic groups are being titrated in this solvent, which confirms the deduction from Conant's data that the most basic group in arginine should be titratable in this solvent.

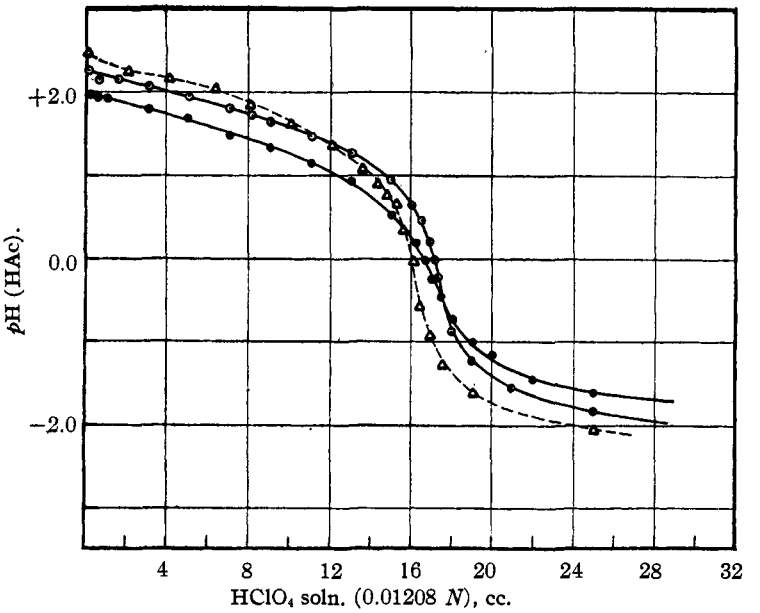

Fig. 2.—⊙, 20 ml. of 0.005 *M* lysine solution; ●, 20 ml. of 0.01 *M* arginine monohydrochloride; △, 20 ml. of 0.005 *M* arginine.

Summary

1. It is pointed out that groups too basic to be titrated in water as solvent, can be titrated in a more acidic solvent, such as glacial acetic acid.

2. The number of equivalents of the dibasic amino acids in a gelatin sample is determined by titration in glacial acetic acid using the hydrogen electrode.[4] For this particular gelatin, the value found was 9.6×10^{-4} equivalent per gram of gelatin.

3. Comparison titrations are reported for lysine and arginine in this solvent, the results being in agreement with those predicted from Conant's measurements on such strong bases as guanidine.

ROCHESTER, NEW YORK RECEIVED JUNE 6, 1935

(3) J. H. Hudson and S. E. Sheppard, *J. Ind. Eng. Chem.*, **21**, 263 (1929).

(4) Using indicator methods, similar results have been obtained by G. F. Nadeau and L. E. Branchen, and are being reported elsewhere.