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On the Isomerism of Hydroxyurea

IV. Acid-base Properties of the Isomers

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It is demonstrated by potentiometric titrations in aqueous solution that the higher melting hydroxyurea exhibits weak acidic properties, $pK_a = 10.6$. The lower melting isomer is a weak base; the conjugate acid has $pK_a = 2.3$. The monobasic properties have been confirmed by titration with perchloric acid in glacial acetic acid. In this medium the complete titration curve was accessible. The weak acidic properties of the higher melting hydroxyurea were also observed in a titration with sodium methoxide in pyridine, although a reproducible titration curve could not be obtained. Comparative studies of methoxyurea revealed that this compound is a weak monoprotic acid; it could be titrated with fair accuracy with sodium methoxide in pyridine.

Previous information on the protolytic properties of the alleged isomers of hydroxyurea has been of a purely qualitative nature. According to Dresler and Stein [1] an aqueous solution of the higher melting hydroxyurea is neutral to litmus paper. The substance has, however, weak acidic properties. Hodges [2] succeeded in preparing hygroscopic metal salts of the type $CH_3O_2N_2K$, $CH_4O_2N_2$, analogous to those formed by hydroxamic acids [3].

The lower melting isomer is reported to be a weak base. Francesconi and Parrozzani [4] have prepared a relatively stable mono-hydrochloride. Their attempts to prepare metal salts of the lower melting isomer, on the other hand, were unsuccessful. Potassium oxyfulminate was obtained instead of the anticipated salt, when hydroxyurea was treated with potassium ethoxide in absolute ethanol.

It was desirable to produce some quantitative evidence for the difference in acid-base properties of the two hydroxyureas since a confirmation of this point would strongly support an explanation on grounds of structural isomerism. For this purpose potentiometric titrations have been carried out in aqueous and non-aqueous media.

RESULTS

Titrations in aqueous solution

Fig. 1a illustrates a titration of the higher melting isomer with hydrochloric acid. The original, freshly prepared solution was neutral, its initial pH being approximately 6.5. The titration curve a is identical with the medium curve c showing that the substance has no detectible basic properties. Here and in the following the term "identical" implies that coincidence can be brought about by a simple translation along the ordinate (ml — axis). In order to find out whether acid-catalyzed rearrangements or decomposition took place the solution was retitrated with sodium hydroxide. The acidic branch of the curve (Fig. 1b) is identical with a. Since the above processes would probably influence the acid-base properties of the system, it may be concluded that they are absent or very slow. In the basic region there is a distinct buffer effect, confirming the weak acidic properties of the higher melting isomer. For the same reasons as above the reversibility was investigated, Fig. 2. To an aqueous solution of the higher melting hydroxyurea was added alkali to pH 10.6 and it was retitrated with acid. The titration curve, Fig. 2a, is identical with Fig. 1b on the alkaline side, but shows a slightly larger acid consumption at

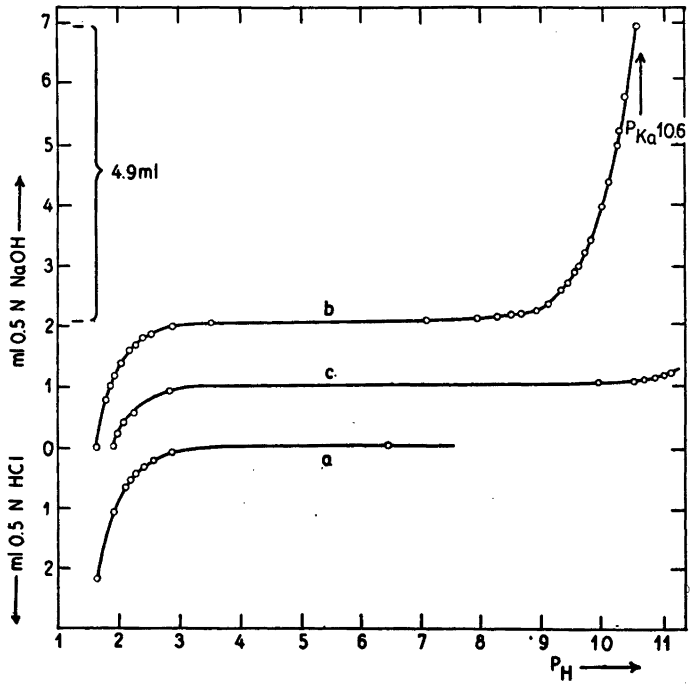

Fig. 1. a. Titration of 4.92 millimoles higher melting hydroxyurea in 40 ml water with 0.5 N hydrochloric acid. b. Retitration with alkali; c. Blank, 40 ml water, added 1 ml 0.5 N hydrochloric acid and titrated.

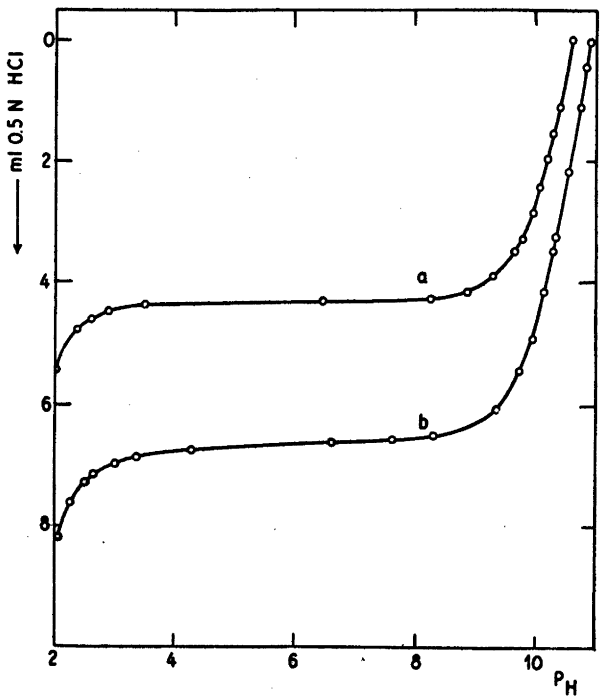

Fig. 2. a. To 4.92 millimoles higher melting hydroxyurea dissolved in 40 ml water was added alkali to pH 10.6 and the solution was titrated with acid; b. Same experiment but the alkalized solution was stored 4 ½ h before retitration.

pH 4—2. This phenomenon is more pronounced in Fig. 2b, in which the same experiment was carried out, except that the alkalized solution was stored for 4.5 h at room temperature before retitration. It may be explained by a base-catalyzed rearrangement to the lower melting hydroxyurea, which has basic properties (*vide infra*), or by a progressive decomposition to *e.g.* ammonia.

The lower melting hydroxyurea was subjected to a similar systematic investigation. An aqueous solution was titrated with hydrochloric acid, Fig. 3 a, and then retitrated with sodium hydroxide, b. The two curves are identical and consequently no irreversible processes seem to occur by change of reaction to the acidic side within the time of observation, *ca.* 45 min. The lower melting hydroxyurea has weak basic properties, as shown by the buffer effect at pH 2—4, *cf.* the medium curve c. There is also a slight buffer effect on the basic side pH 8—10. This does not necessarily mean that the lower melting isomer has acidic properties. It is known that the substance, when heated in alcoholic solution, is partially rearranged to the higher melting hydroxyurea, which has acidic properties *(vide supra)*, and partially decomposed. Similar reactions may proceed slowly in aqueous solution at room temperature, possibly catalyzed by acid or base.

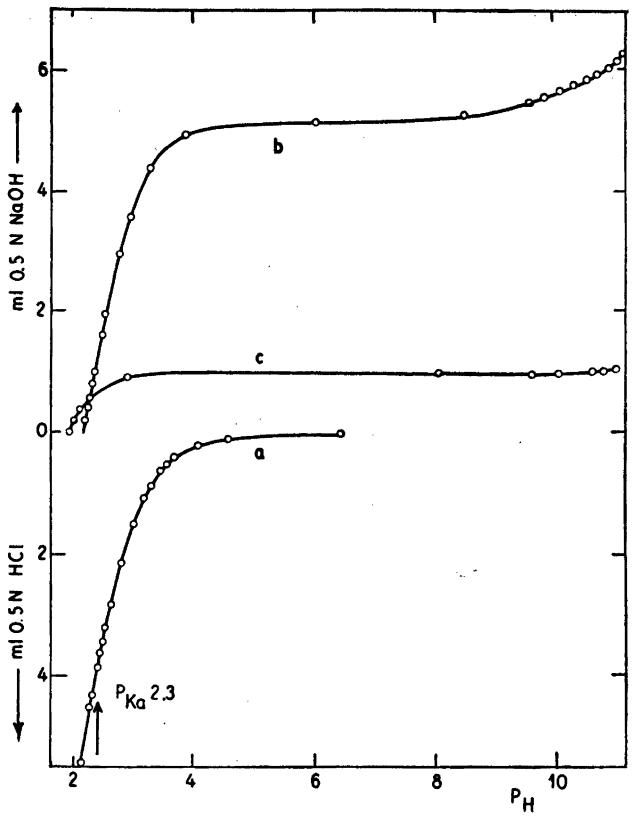

Fig. 3. a. Titration of 4.20 millimoles lower melting hydroxyurea in 40 ml water with 0.5 N hydrochloric acid; b. retitration with alkali; c. Blank; to 40 ml water was added 1 ml 0.5 N hydrochloric acid and the solution titrated.

For the final general discussion of the structural problem the essential result of the above experiments is that the higher melting hydroxyurea is a weak acid and the lower melting one is a weak base. The dissociation constants are so small that a complete titration to the equivalence point is impossible in aqueous medium and the method is inapplicable as an analytical tool. This is in accordance with conductivity measurements reported previously [5]. A rough estimation of the dissociation constants may, however, be attempted. Let it be assumed that both isomers require 1 equivalent titrant per mole for complete "neutralization". The quantity of the higher melting isomer titrated in Fig. 1 represents 4.92 millimoles, which would require 4.92 ml 0.5 *N* sodium hydroxide for "half neutralization". The corresponding pH read on the curve, Fig. 1b, is *ca.* 10.6. The approximate dissociation constant for the higher melting isomer is consequently $K_a = 10^{-10.6}$. In the titration given in Fig. 3 4.20 millimoles of the lower melting isomer was taken. On the assumption

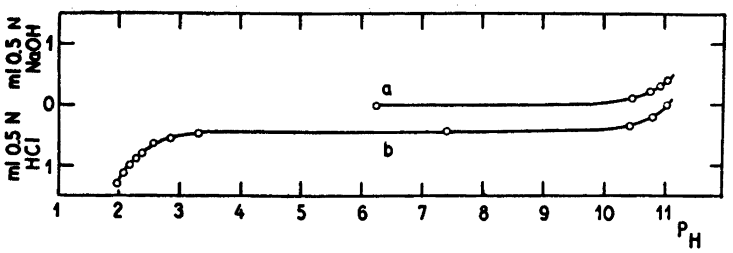

Fig. 4. a. Titration with alkali of 4 millimoles methoxyurea in 40 ml water; b. retitration with acid.

that it is monoprotic, this quantity requires 4.20 ml 0.5 *N* hydrochloric acid for "half neutralization". The corresponding pH is 2.3. Thus $K_a = 10^{-2.3}$ is a probable dissociation constant of the hydroxyuronium ion formed by the lower melting isomer.

As an aid in a structural interpretation of the observed protolytic properties of the hydroxyureas the related substances methoxyurea and urea have been titrated with the same technique. Experimental points are given in Figs. 4—5, and for convenient comparison all the characteristic titration curves, normalized to 4 millimoles substance, have been plotted in Fig. 6, including the blank curve for the solvent (aq). The urea curve (u) is identical with that of the solvent, whereas methoxyurea (m) shows a slight buffer effect at pH 10—11, and should therefore be a very weak acid, much weaker than the higher melting hydroxyurea (h). The weak acidic properties of methoxyurea have been confirmed by titration in non-aqueous medium, *vide infra*. From the results summarized in Fig. 6 it appears that the acid-base properties of the hydroxyureas cannot be attributed to the urea torso, but must be characteristic of the hydroxylamine part of the molecule or at least induced by it. A normal hydroxylamine structure with a free hydroxy group would explain the weak acidic properties of the higher melting isomer, whereas an amine oxide structure would account for the basic properties of the lower melting hydroxyurea. The existence of only one methoxyurea and the much weaker protolytic properties of that compound fit well into the picture. There are, however, other possibilities, and the whole structural problem including the localization of the acid-base properties will be discussed in a larger context in a final paper.

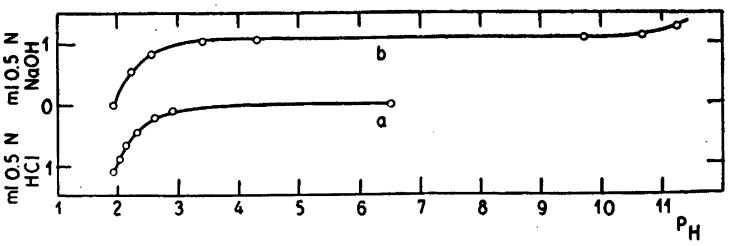

Fig. 5. a. Titration with acid of 4 millimoles urea in 40 ml water; b. retitration with alkali.

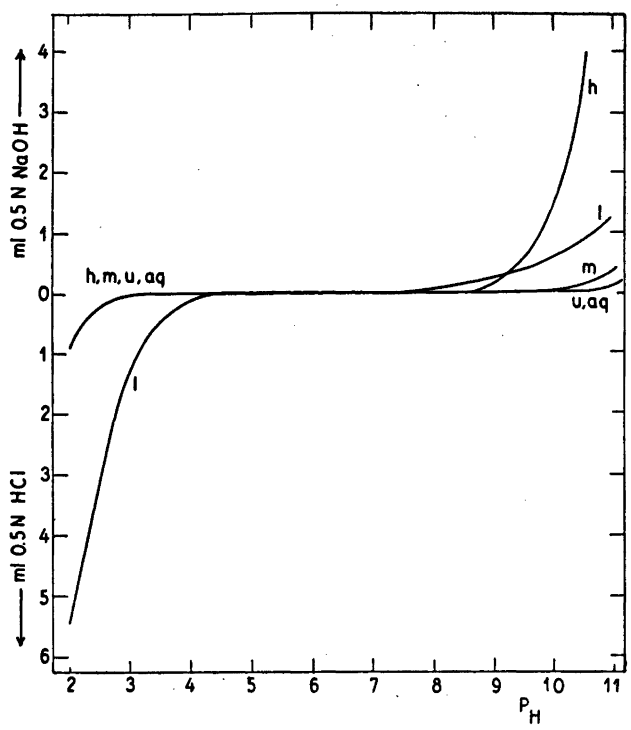

Fig. 6. Titration curves fig. 1—5 converted to 4 millimoles substance.
h. higher melting hydroxyurea l. lower melting hydroxyurea.
u. urea m. methoxyurea aq. solvent blank (water).

Titrations in non-aqueous media

Acid-base titrations in various non-aqueous solvents have in recent years attracted considerable interest because in this way the simple, visual or potentiometric titration technique can often be extended to acids and bases, which are too weak to be titrated in water. Thus in glacial acetic acid many weak bases will be sufficiently strong to permit titration with perchloric acid. A review of this method has recently been given by Riddick [6]. Analogously weak acids may often be titrated in basic media such as pyridine and various aliphatic amines with sodium methoxide as a titrant. For a review of this method see ref. 7.

As discovered in the first part of this paper the hydroxyureas are typical examples of a weak acid and a weak base respectively, the equivalence points of which are beyond reach in aqueous solution. It was, therefore, interesting to try whether more precise and complete information could be obtained in non-aqueous media.

It was found that the lower melting hydroxyurea was soluble and could be readily titrated potentiometrically in glacial acetic acid, using perchloric acid

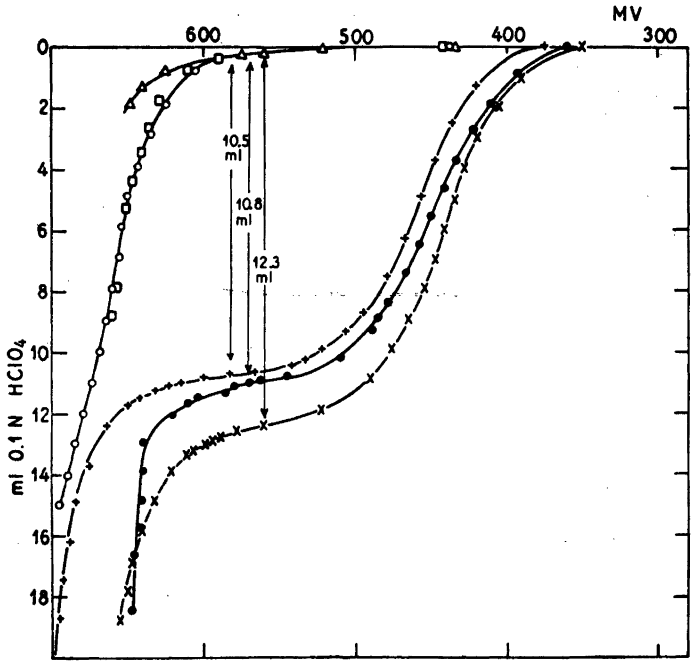

Fig. 7. Titrations with 0.1 N perchloric acid in glacial acetic acid; 0.100 g substance, 40 ml solvent.

× *lower melting hydroxyurea, immediately after isolation.*
● *same sample after 1 week storage at −10° C.*
\+ *same sample after a total of 2 weeks storage at −10° C and 48 h at 23° C.*
○ *higher melting hydroxyurea, immediately after isolation.*
□ *methoxyurea.*
△ *solvent blank.*

in the same solvent as a titrant. A typical titration curve is shown in Fig. 7, notation ×. The first part of the curve, at approximately 350 to 450 MV, corresponds to the incomplete titration curve, Fig. 3 a, obtainable in aqueous medium. In glacial acetic acid the titration can be carried to an end, the lower melting isomer behaves as a monoprotic base. The experimental equivalent weight is 81.3 (1 gram comsumes 12.3 ml 0.1000 *N* perchloric acid). This is slightly more than the theoretical value, 76.0, but leaves little doubt that the substance is monoprotic, which was assumed in the estimation of the aqueous dissociation constants above. For a computation of dissociation constants from the acetic acid data it is necessary to work out a standard curve, relating potential in acetic acid to dissociation constant in water for the particular electrode system, such as carried out by Veibel *et al.*[8] This was not done in the present case.

The high experimental equivalent weight may be due to the lability of the substance (*cf.* ref. 9). This point was further investigated by following the variation of the equivalent weight with time. The solid was stored over

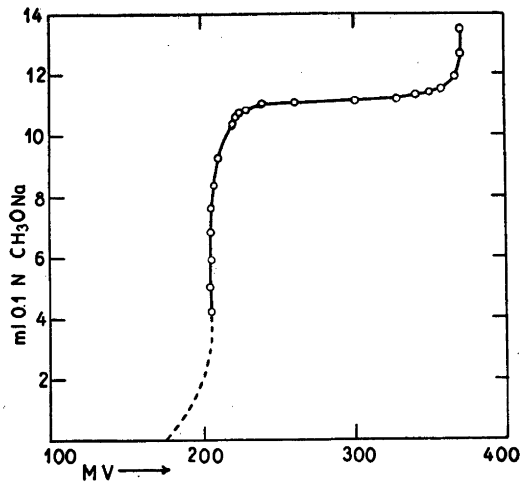

Fig. 8. Titration of 0.100 g methoxyurea in 50 ml "pyridine-bases" with 0.1 N sodium methoxide.

phosphorus oxide at —10° and at room temperature. Further details are given in Fig. 7. The equivalent weight increases with time even when the substance is stored at low temperature. After the last titration, the rest of the sample was stored at room temperature for 2 weeks; the decomposition was then obvious from its appearance.

The titration curves of the higher melting hydroxyurea (○) and of methoxyurea (□) in Fig. 7 seem to suggest that these compounds, besides being weakly acidic, have extremely weak basic properties, *cf.* the solvent blank (△).

The weak acidic properties of the higher melting hydroxyurea, observed in aqueous solution should be more pronounced in basic solvents. Potentiometric titration was attempted in "pyridine-bases", a commercial mixture of homologues successfully employed by Heiz [7] in this laboratory for the titration of barbituric acids. The titrant was sodium methoxide in benzene-methanol as suggested by Fritz and Lisicki [10], whose general procedure was also followed. A glass/antimony electrode combination gave poorly defined potentials. Every addition of titrant caused a change in voltage reading, but the pointer drifted slowly back again, without reaching an equilibrium position. By taking the maximum readings, however, a fairly reproducible titration curve could be obtained for the primary standard, benzoic acid, and for methoxyurea. The latter is given in Fig. 8. The equivalent weight 89.3 agrees satisfactorily with the theoretical value, 90.1. The acidic properties of this substance, faintly suggested in Fig. 6, m, have thus been confirmed.

The higher melting hydroxyurea could not be titrated reproducibly, but it was beyond doubt that it consumed the titrant in accordance with its acidity in aqueous solution, Fig. 6, h.

A calomel/antimony electrode combination suffered from the same instability as the above system. In fact the lack of adequate electrode systems is often a serious limitation to titrations in non-aqueous solvents. There is in this field a pronounced tendency toward an uncritical usage of conventional aqueous electrodes such as glass and calomel electrodes. It must be admitted,

that these arrangements sometimes serve analytical purposes quite well, but from a physico-chemical points of view it would be more satisfactory to set up specific electrode cells based on the relevant solvent and not on water. But the problem of electrode instability in non-aqueous solvents will perhaps eventually be most effectively overcome by the promising new technique of high-frequency titration, in which immersed electrodes are avoided altogether[11].

EXPERIMENTAL

Materials. The preparation of the *hydroxyureas* and of *methoxyurea* have been described in previous papers [9,14].

Glacial acetic acid. To an analytical grade product was added a calculated amount of acetic anhydride in order to reduce the water content to less than 0.2 %. The percentage was checked according to the method of Karl Fischer. Excess of acetic anhydride was carefully avoided, because it might acetylate the compounds to be titrated.

"Pyridine-bases". A commercial mixture of pyridine homologues was carefully dried over potassium hydroxide and distilled. The fraction distilling at 114—150° C was used.

Methanol and *benzene* were dried and distilled over sodium.

Titrations in aqueous solution were carried out in a nitrogen atmosphere in a closed titration vessel, using a glass electrode and a "saturated calomel" electrode. The potential was determined with a Radiometer tube potentiometer. A standard curve voltage *vs.* pH was worked out on the basis of conventional standard buffer solutions. The titration vessel was fitted with a magnetic stirrer.

Titrations in glacial acetic acid. Titration vessel and electrode combination as above. The titrant, 0.1 *N* perchloric acid, was prepared essentially as recommended by Markunas and Riddick [12]. The exact normality was determined against potassium biphthalate. All volume readings were corrected to 0.1000 *N* titrant, taking into account also possible deviation of titration temperature from that of the standardization (thermal expansion coefficient of glacial acetic acid 0.0011).

Titration in "pyridine-bases". Glass/antimony electrode combination. Titrations in a closed vessel with nitrogen blanketing and magnetic stirring. 0.1 *N* sodium methoxide in benzene-methanol was prepared as indicated by Vespe and Fritz [13]. The exact normality was determined against benzoic acid as a primary standard.

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