REACTION OF UREA WITH ETHYL ACETOACETATE

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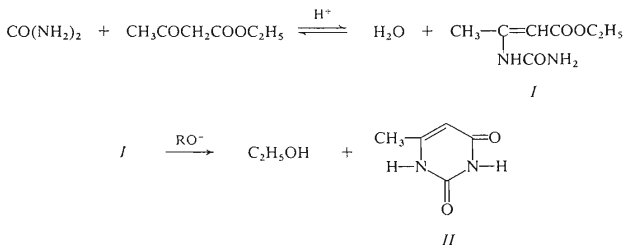*Organic Chemistry Department,*
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Rate and equilibrium constants of the reaction of urea with ethyl acetoacetate have been measured in 99·4 and 98·9% (by vol.) aqueous methanol, and rate constants of hydrolysis of the formed ureide have been measured in water and water-methanol mixtures. The reaction is subject to general acid catalysis. The subsequent cyclization of the ureide to 6-methyluracil is subject to specific base catalysis and is by 3 orders of magnitude faster in water than in methanol at the same concentration of the lyate ion. The both dissociation constants of 6-methyluracil have been measured both in water and in methanol, and structure of the monoanion has been determined.

Many uracil derivatives block the enzymes in plant tissues which catalyze photosynthesis. Therefore, they act as efficient selective herbicides[1]. A number of biologically active uracil derivatives along with their preparation methods are described in patent literature. Four methods are used for these syntheses[2], differing in the way of preparation of the ureide which is further cyclized in basic medium to give the uracil derivative. Although the herbicides based on uracil are produced industrially, so far no kinetic studies of their preparation have been published. On the contrary, there are papers[3-5] dealing with cyclization kinetics of saturated ureide derivatives leading to the biologically inactive uracil dihydro derivatives and with the respective reverse reaction.

The presence of double bond in ureide and uracil changes fundamentally their reactivity compared with similar saturated derivatives. The uracil dihydro derivatives undergo ring opening both in acid and basic media, and the formed ureide is again cyclized in acid medium (the reaction is reversible), being practically not hydrolyzed. On the contrary the uracil *II* is very stable both in acid and in alkaline media, but the ureide *I* is practically immediately hydrolyzed in mineral acid medium to give urea and acetoacetate (Scheme 1).

$$CO(NH_2)_2 \ + \ CH_3COCH_2COOC_2H_5 \ \underset{}{\overset{H^+}{\rightleftharpoons}} \ H_2O \ + \ CH_3{-}\underset{\underset{NHCONH_2}{|}}{C}{=}CHCOOC_2H_5$$

I

$$I \ \xrightarrow{RO^-} \ C_2H_5OH \ + \ \text{[structure of 6-methyluracil, II]}$$

II

Scheme 1

In this work kinetics of formation and reverse hydrolysis of the ureide I and its cyclization to uracil II are studied. Besides that the dissociation constants of the compound II were determined in water and methanol.

EXPERIMENTAL

Reagents

Ethyl ester of 3-ureido-2-butenoic acid (I) was prepared by condensation of urea and ethyl aceto-acetate[6] (m.p. 148—149°C). 6-Methyluracil (II) was prepared by cyclization of the ureide I in aqueous sodium hydroxide[6] (m.p. 325—326°C). Ethyl ester of 3-(N′-tert-butylureido)-2-butenoic acid (III) was prepared by condensation of ethyl acetoacetate with N-tert-butylurea (prepared from tert-butylalcohol and urea[7]) in benzene with catalysis by 4-toluenesulfonic acid in the yield 44%. M.p. 116—117·5°C. For $C_{11}H_{20}N_2O_3$ (228·2) calculated: 57·87% C, 8·22% H, 12·27% N; found: 57·69% C, 8·76% H, 11·98% N. 3-Tert-butyl-6-methyl-1,2,3,4-tetrahydropyrimidine-2,4--dione (IV) was prepared[2] by cyclization of the ureide III in 2M-CH_3ONa. Yield after crystalliza-tion from water was 54%, m.p. 136·5—137°C. For $C_9H_{14}N_2O_2$ (182·2) calculated: 59·30% C, 7·74% H, 15·37% N; found: 59·33% C, 7·90% H, 15·36% N. The other reagents used were commer-cial chemicals of p.a. purity grade.

Kinetic Measurements and Determination of Dissociation Constants

For the measurements the stock solutions of the ureide I and uracils II and IV were prepared. The fresh ureide I solution was used for few days only. Methanolic hydrochloric acid was prepared by introducing gaseous hydrogen chloride into methanol, and its concentration was checked every second day; fresh acid was prepared every week. The measurements were carried out spectrophotometrically with a VSU-2P Zeiss apparatus in a quartz cell at 25°C.

Hydrolysis of the ureide I. 0·2 ml 1 . 10^{-3}M ureide I solution resp. one drop 5 . 10^{-3}M solution was added in the cell containing water or aqueous methanol, hydrochloric acid or chloroacetate buffer (ionic strength adjusted at 0·5 by addition of KCl) or urea and hydrogen chloride (total volume 2 ml). The absorbance decrease at 274 nm was followed.

Formation of the ureide I. 0·3 ml 5 . 10^{-3}M ethyl acetoacetate was added in the mixture of 1·9 ml aqueous methanol, hydrochloric acid and urea. The absorbance increase at 274 nm was followed.

Cyclization of the ureide I. 0·2 ml 1 . 10^{-3}M ureide I stock solution was added in the cell con-taining 2 ml aqueous carbonate buffer or mixture of secondary and tertiary sodium phosphates (ionic strength 1·5), and the absorbance decrease at 265 nm was followed. At the same wavelength the cyclization kinetics were followed in 0·1 to 1M-CH_3ONa.

Measurement of the dissociation constants. The first dissociation constant of the uracil II in water was measured in borax buffer at the ionic strength 0·01 to 0·04 (ref.[8]). The dissociation constant value was calculated from Eq. (1) (ref.[8]),

$$- \log K_{HA} = p(a_H^+ . f_{Cl(-)}) - \log I + \log (f_{AH} . f_{Cl(-)}/f_{A(-)}) \qquad (1)$$

$$pK_a = - \log I + H_- \qquad (2)$$

where K_{HA} is the measured dissociation constant, $p(a_{H^+}f_{Cl^{(-)}})$ is the value of acidity function of borax buffer[8], f is activity coefficient of individual component, and I stands for the concentration ratio of the conjugated base and acid of the substance measured, which was obtained spectrophotometrically. The second dissociation constant of the uracil *II* was measured in aqueous sodium hydroxide solutions (0·2 to 3M-NaOH), and the pK_a value was computed from Eq. (2), using the published[9] H_- values.

Dissociation constant of the tert-butyl derivative *IV* was measured in aqueous sodium hydroxide solutions (0·01 to 0·05M NaOH). The pK_a value was calculated[10] from Eq. (3) where K_w is ionic product of water.

$$pK_a = - \log I + pK_w - \log \left[OH^- \right] \qquad (3)$$

Dissociation constants in methanolic sodium methoxide were measured within the concentration ranges 0·001 to 0·01M CH_3ONa (the first dissociation of the compound *II*); 0·1 to 5M CH_3ONa (the second dissociation of the compound *II*); 0·002 to 0·04M-CH_3ONa (dissociation of the compound *IV*). The pK_a values of the first dissociation step were calculated from Eq. (4) where K_M is the ionic product of methanol (from ref.[11] $pK_M = 16.916$). The pK_a values of the second dissociation step were obtained from Eq. (5), the values of ref.[12] (obtained from the dissociation constants of the substituted indoles) being taken for the acidity function H_M.

$$pK_a = - \log I + pK_M - \log \left[CH_3O^- \right] \qquad (4)$$

$$pK_a = - \log I + H_M \qquad (5)$$

The NMR spectrum of the ureide *I* was measured in $CDCl_3$ at 50°C using a Tesla BS 487 A apparatus at 80 MHz. Hexamethyldisiloxane was used as internal standard. The found chemical shifts (τ) were: −0·60 (NH); 4·69 (NH₂); 5·23 (=CH—); 5·90 (CH₂—CH₃); 7·67 (=C—CH₃) and 8·78 (—CH₂—CH₃).

RESULTS AND DISCUSSION

Hydrolysis rate of the ureide *I* in diluted hydrochloric acid solutions is of the first order in the proton concentration, and in chloroacetate buffers the dependence of the rate constants on the chloroacetic acid concentration is linear. Hence the ureide hydrolysis is subject to general acid catalysis, and k_{obs} is defined by Eq. (6). The rate constant of the proton-catalyzed reaction $k_{H^+} = 1·01 \pm 0·10 \, l \, mol^{-1} \, s^{-1}$ was determined by measurements in diluted hydrochloric acid and chloroacetate buffers. The proton concentration in the chloroacetate buffers was calculated from the measured pH value and the proton activity coefficient calculated[8] from Eq. (7). The value $(4·0 \pm 0·2) . 10^{-2} \, l \, mol^{-1} \, s^{-1}$ was calculated for k_{AH}. Mechanism of the acid

$$k_{obs} = k_{H^+} \left[H^+ \right] + k_{AH} \left[AH \right] \qquad (6)$$

$$\log f = -0·5 \sqrt{J} / (1 + 1·5 \sqrt{J}) \qquad (7)$$

catalyzed ureide hydrolysis is represented in Scheme 2, where HA stands for H_3O^+ or hydrochloric acid.
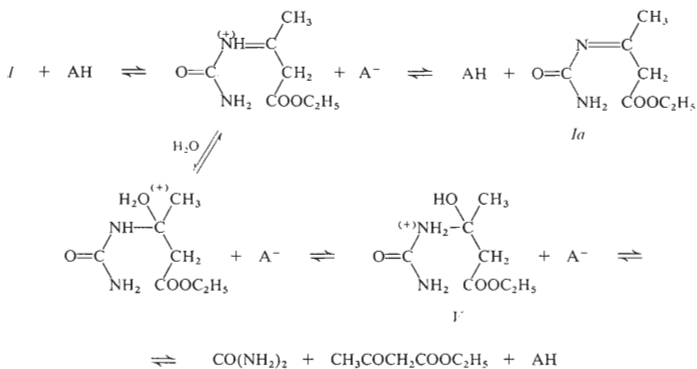
Scheme 2

From the NMR spectrum in $CDCl_3$ it was found that the ureide has the structure I, whereas the presence of the second tautomer Ia was not proved. Suppose the structure I predominates in water, too, either protonation of the compound I or reaction of the protonated carbinolamine with the conjugated base A^- can be rate limiting. In the former case the reaction rate depends on the ureide (I) concentration and acid concentration; in the latter case concentration of water makes itself felt in addition to it. The hydrolysis kinetics was followed in mixtures water–hydrochloric acid–methanol, and the logarithms of rate constants were compared with the values of the acidity funciton H_0 for aqueous methanolic hydrochloric acid[13,14] to decide between the above two alternatives. The dependences of both $\log k$ and H_0 function on the methanol concentration are practically the same up to about 90% (by vol.) methanol (Fig. 1), but at higher concentration of methanol the increase in $\log k$ is gradually slower than that of H_0. In 99·6% methanol the $\log k$ value is lower than that in 99·0% methanol. It means that water is highly significant kinetically in this region. The rate-determining step thus consists either in the reaction of the protonated carbinolamine with base or addition of water to the protonated ureide, which would indicate a change in the rate-determining step in this region. The experiments carried out in the media 99·35% (by vol.) and 98·90% (by vol.) CH_3OH with the buffer urea–urea hydrochloride showed that the hydrolysis rate constant increases with

increasing buffer concentration* (Fig. 2). This indicates that in this region, too, the reaction is subject to general acid catalysis, and the rate-determining step consists in the reaction of the protonated carbinolamine (V) with the conjugated base. Extrapolation of k_{obs} (Fig. 2) to the zero buffer concentration gives the $k_{H^+}[H^+]$ values. From these values and k_{H^+} obtained from direct measurements in the presence of hydrochloric acid it was possible to calculate the proton concentration wherefrom pK_a of the protonated urea in 99·35% CH_3OH (2·72) and in 98·90% CH_3OH (2·55) could be computed. These values are by about two units higher than those determined in water[15]. The corresponding increase caused by solvent is about 0·3 to 0·5 in the case of the true Hammett bases[13,14].

The second alternative is that the pK_a value of urea in methanol is about 1 (in accordance with the Hammett bases) and lowering of the ureide hydrolysis rate in the presence of the urea buffer (in contrast to the experiments without addition of urea, Figs 1 and 2) is due not to a strong decrease of the proton concentration but to a specific retarding effect of urea. However, this alternative is contradicted by that the specific influence of mere 0·03M total urea concentration would have to cause a 5 to 10 fold slowing down of the reaction, and that the ratio $[I]$/[aceto-acetate] in the equilibrium mixture is directly proportional to the first power of urea concentration and inversely proportional to water concentration in accord with the stoichiometric
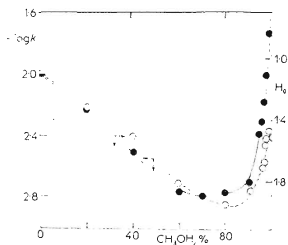

FIG. 1

Dependence of H_0 (●) resp. log k (s^{-1}) of the Ureide I Hydrolysis (○) in 0·01M-HCl on Methanol Concentration at 25°C
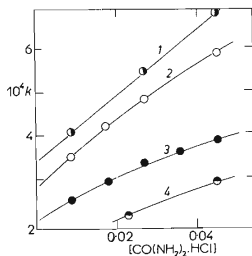
FIG. 2

Dependence of Rate Constants k (s^{-1}) of the Ureide I Hydrolysis in Aqueous Methanol on Concentration and Composition of the Buffer Urea–Urea Hydrochloride at 25°C

Ratio of the buffer components and content of water in methanol: 2 : 1, 0·36M (1); 4 : 1, 0·61M (2); 4 : 1, 0·36M (3); 15 : 1, 0·61M (4).

*   The reaction is shifted in favour of the hydrolysis products so strongly that, even at the highest used urea concentrations (0·7M), 85% of the ureide was hydrolyzed.

equation. Specific influence of urea on reactivity of the individual components would have to make itself felt in the equilibrium constants of the reaction. At the highest urea concentration in 99·35% CH_3OH the dependence has a decreasing slope (Fig. 2). It can be due to a partial change of the rate-determining step (water addition to the protonated ureide) or to a partial change of medium caused by high urea concentration.

With respect to high inclination of the ureide I to hydrolyze to urea and acetoacetate, the equilibrium constant of this reaction (Scheme 1) was measured in methanol medium containing only a small amount of water (0·65 to 1·15% by vol.) and relatively high urea concentration (0·32 to 0·68M). Even under these conditions the ureide concentration in the equilibrium mixture was only 4·5 to 15%. Ratio of ureide to acetoacetate concentrations was determined from the absorbance increase at 275 nm (corresponding to formation of the ureide I) and from the separately determined absorbance of the pure ureide. The experimental conditions for determination of the ratio $[I]/[\text{acetoacetate}]$ were the same as those for kinetic measurements of this reaction.

As the reaction is reversible, the k_{obs} value represents a sum of the rate constants of formation and hydrolysis of the ureide. Therefore, both the rate and the equilibrium constants can be measured by following the reaction in the both directions. The measured reaction is accompanied by a still slower irreversible reaction (decomposition of acetoacetate?). The error in k_{obs} (due to the side reaction) is smaller by about one order of magnitude in measurement of the ureide hydrolysis. The k_{obs} value thus obtained was then used for calculation of the absorbance increase (275 nm) in the reaction of acetoacetate with urea carried out under the same reaction conditions.

Value of the ratio $[I]/[\text{acetoacetate}]$ is directly proportional to the first power of urea concentration and inversely proportional to water concentration. The equilibrium constant is defined by Eq. (8), its value being $0·092 \pm 0·004$.

$$K = [I][H_2O]/[CH_3COCH_2CO_2C_2H_5][CO(NH_2)_2] \qquad (8)$$

Rate of the base-catalyzed cyclization of the ureide I to uracil II in aqueous buffer solutions is directly proportional to the hydroxyl ion concentration, being independent of the carbonate buffer concentration, and it shows a mild increase with increasing phosphate buffer concentration. This increase can be due to a specific influence of phosphate ions on the activity coefficients of the starting substances and the activated complex. This reaction is subject to specific base catalysis. The reaction mechanism can be formulated in two ways (Scheme 3). Value of the bimolecular constant $k_2 = 5·21 \text{ mol s}^{-1}$, calculated as the quotient of experimental constant k and hydroxyl ion activity, is by about two orders of magnitude greater than the rate constant of alkaline hydrolysis of ethyl propionate and ethyl acrylate[16]. Hence it follows that saponification of the ester group of the ureide I is practically insignificant during cyclization. The cyclization $I \rightarrow II$ was further studied in methanolic sodium methoxide (0·1 to 1·0M-CH_3O^-). The experimental rate constants k increase at
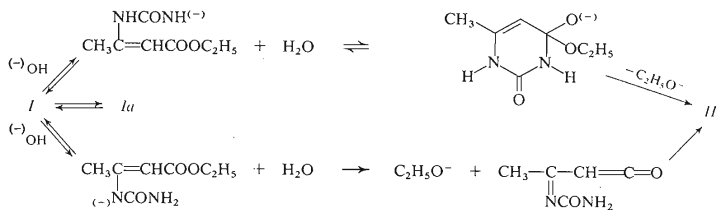
SCHEME 3

higher methoxide concentrations somewhat faster than $[CH_3O^-]$. However, dependence of the logarithms of the rate constants on the H_M function[17] is linear with the slope equal to unity. The rate constant k_2 was calculated by dividing the experimental rate constant k by the alkoxide concentration at 0·1 to 0·2M-CH_3O^-. Its value $(5·8 . 10^{-3}\,l\,mol^{-1}\,s^{-1})$ is smaller by about three orders of magnitude than the value of the analogous rate constant measured in aqueous sodium hydroxide. A part of the difference between the cyclization rate constants in aqueous and methanolic media is probably due to that the dissociation degree of the ureide I in aqueous sodium hydroxide is greater than in methanolic sodium methoxide of the same concentration. Such solvent effects are usual[18] and were observed also in the measurements of the dissociation constants of the uracil II (Table I).
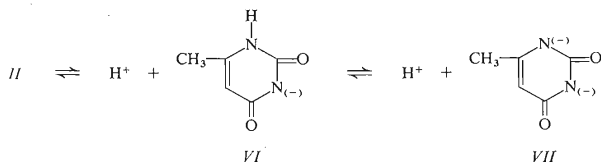
SCHEME 4

TABLE I

Dissociation Constants of the Compounds II, IV and VI in Water and in Methanol at 25°C

Compound	H_2O	CH_3OH	λ_{anal}, nm
II	9·73 ± 0·01	13·74 ± 0·08	285; 290
IV	11·35 ± 0·03	15·00 ± 0·02	295
VI	14·07 ± 0·01	17·32 ± 0·02	290; 295

In borax and carbonate buffer solutions (pH 9−11) the uracil *II* is transformed into the monoanion *VI* (Scheme 4) having λ_{max} 285 nm. In aqueous sodium hydroxide medium ([NaOH] > 0·05M) gradual absorbance decrease at 285 nm is observed, and a new absorption band is formed at 276 nm corresponding to the dianion *VII*. Both in buffer solutions and in aqueous sodium hydroxide the spectra taken with the Unicam SP 800 apparatus showed well developed isosbestic points. The dissociation constants values are given in Table I. The first proton can be split from 1- or from 3-position. For the sake of determination of the monoanion structure the dissociation constant was found also for the tert-butyl derivative *IV* containing a dissociable proton only in 1-position. Spectroscopic investigation of this dissociation reaction again showed isosbestic points. The pK_a value of the compound *IV* is greater than pK_a of the uracil *II* by 1·5 units (Table I). Therefrom it follows that the proton is split off from the uracil *II* at a position different from that in the case of the tert-butyl derivative *IV*. Similar situation was encountered also in the measurements of dissociation constants of the compounds *II* and *IV* in methanol (Table I).

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