KINETICS OF HYDROLYSIS AND REARRANGEMENTS OF S-ACYLTHIOURONIUM SALTS

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Kinetics of transformation of S-acetyl- and S-benzoylisothiouronium chlorides have been followed, and its reaction products in dilute hydrochloric acid media and aqueous buffers have been identified. In dilute hydrochloric acid the reaction rate is pH-independent, and the acetyl derivative reacts 8 times as rapidly as the benzoyl derivative. In acetate and phosphate buffers the decomposition rate of the both derivatives increases linearly with the buffer concentration. In acetate buffers the reaction rate is pH-independent, and acetate ion reacts as a nucleophile. In phosphate buffers the rate increases with increasing pH. The reaction catalyzed by the basic buffer component produces thiourea and carboxylic acid, that catalyzed by hydroxyl ion produces N-acetyl- or N-benzoylthiourea. The solvolysis rates of the both S-acyl derivatives depend on percent composition of water–methanol solvent. The maximum solvolysis rate is reached in methanol with 25% (v/v) water.

Transferase enzymes can transfer certain groups from a donor to an acceptor[1]. Special transferases – transacylases – can transfer an acyl group; a typical example is vitamin biotin[2,3]. Therefore, Bruice[2,3] chose S-benzoylthiourcas (inclusive of S-benzoylisothiobiotin) as simple models for studies of transfer of acyl group in this type of enzymes. At low pH values, hydrolysis of S-benzoyl group of the thiouronium salt represents practically the only reaction. With increasing pH increases significance of rearrangement of benzoyl group to nitrogen atom, the rearrangement being almost quantitative with non-cyclic compounds in neutral media. In basic media elimination reactions predominate[2,3].

Transfer of aliphatic acyl groups is more usual in natural processes. Therefore, we have studied kinetics and mechanism of transformations of S-acetylthiourea in dilute hydrochloric acid and buffer solutions. We have found that there is substantial difference between S-acetyl and S-benzoyl derivatives not only in the reaction rates but also in the proper reaction course especially in neutral (buffer solutions) and basic media. For quantitative determination of reactivity differences of the two acyl derivatives we also have studied the reactions of S-benzoylthiourea under the same conditions.

EXPERIMENTAL

Reagents

Acetyl chloride was prepared in 90% yield by reaction of acetanhydride and benzoyl chloride; the fraction boiling at 51–52°C was taken[4]. S-Acetylisothiouronium chloride (*I*) was prepared from acetyl chloride and thiourea in acetone; yield 91%, m.p. 101–103°C (ref.[5], m.p. 197°C). S-Benzoylisothiouronium chloride (*II*) was prepared from benzoyl chloride and thiourea in acetone solution; yield 85%, m.p. 130–132° in accordance with ref.[3]. N-Acetylthiourea (*III*), m.p. 168–169°C, was prepared in 35% yield by acetylation of thiourea with acetanhydride and subsequent partial hydrolysis of the product with sulphuric acid[6]. N-Benzoylthiourea was prepared by reaction of aqueous ammonia with benzoylisothiocyanate (prepared from benzoyl chloride and ammonium rhodanide[7]). Yield 80%, m.p. 168–170°C (ethanol) in accordance with ref.[8].

Kinetic Measurements

Kinetics of reactions of compounds *I* and *II* were followed with the use of a Zeiss Specord spectrophotometer at the wavelength 237 nm (absorbance increase) and, in case of the benzoyl derivative *II*, also at 263 nm (absorbance decrease). For each kinetic run of reactions of *II*, fresh stock solution was prepared (about 10^{-3} mol l^{-1} in 10^{-3} mol l^{-1} hydrochloric acid). One drop of this solution was added to 2 ml reaction solution placed in 1 cm quartz cell temperated at 25°C. As the solvolysis half-time of acetyl derivative *I* was about 4 min even in methanolic hydrochloric acid, the stock solutions could not be prepared. The following procedure was adopted: a crystal of the acetyl derivative *I* was added into the temperated solution placed in quartz cell, the solution was mixed, and the absorbance–time dependence was recorded immediately. As the acetyl derivative *I* is decomposed even in solid state, a fresh sample was prepared each day before the measurements and was kept in dessiccator. Ionic strength $I = 1{\cdot}0$ was adjusted by addition of potassium chloride in the cases of kinetic experiments in buffer solutions.

Product Identification under Conditions of Kinetic Experiments

The acetyl derivative *I* (36·50 mg) was dissolved in 100 ml of the reaction solution. After about ten reaction half-lives an aliquot portion was ten times diluted with water and its spectrum measured within the region 210 to 370 nm. In the experiments carried out in sodium carbonate and hydroxide solutions, the mixture was acidified to pH 6 after about 7 s from dissolution of *I*, because the N-acetylthiourea (*III*) possibly formed is hydrolyzed in basic media. At the same time spectra were measured of the prepared mixtures of thiourea and acetyl derivative *III* and sodium acetate to obtain the calibration straight lines for determination of concentrations of thiourea and its acetyl derivative (*III*). Only thiourea was proved spectroscopically in solutions of hydrochloric acid, sodium hydroxide and carbonate. The same was proved also in the experiments carried out on preparative scale: 5 g acetyl derivative *I* was dissolved in 50 ml solution of 10^{-1} mol l^{-1} hydrochloric acid (or 0·5 mol l^{-1} sodium carbonate or hydroxide) with stirring. The mixture was neutralized and vacuum-evaporated. The solid residue was boiled with 50 ml ethanol, filtered, the filtrate was distilled, and the distillation residue identified as thiourea.

The reactions in phosphate and borax buffers give thiourea along with its acetyl derivative *III*. Amount of the latter was determined from spectral records with the use of the calibration straight line (Table I).

Evidence of Nucleophilic Catalysis in Hydrolysis of *I* in Acetate Buffers

a) 3·1 g (0·02 mol) acetyl derivative *I* was dissolved in 100 ml saturated aniline solution (about 4 mol l^{-1}). After 1 h, 50 ml aqueous solution was added containing 2·4 g anhydrous sodium acetate, and the separated solid was filtered off, dried and weighed.

b) Solution of 2·4 g anhydrous sodium acetate in 50 ml water was treated with 3·1 g acetyl derivative *I*. After 0·5 min, 100 ml saturated aniline solution was added. After 1 h the separated solid was filtered off, dried and weighed. The same procedure was repeated, aniline solution being added after 1, 2, 3 and 4 min. All the products were identified as acetanilide by their melting point. The amount of acetanilide remaining in the filtrates was 0·85 to 0·95 g and was determined gravimetrically.

Determination of Methanolysis-Hydrolysis Ratio of the Benzoyl Derivative in Methanol-Water Mixtures in Acid Media

The benzoyl derivative *II* (7·3 g, 0·033 mol) was added to 50 ml 10^{-3} mol l^{-1} hydrochloric acid in water-methanol mixture (25 : 75 v/v). The solution was left to stand at room temperature for 1 h. Then it was diluted with 400 ml water, neutralized to pH 7 with aqueous sodium carbonate solution, and extracted four times with 100 ml benzene. The benzene extract was dried, concentrated and adjusted to 10 ml volume. The methyl benzoate content was determined by gas chromatography. The aqueous solution after extraction was concentrated to 25 ml and acidified to pH 1. The precipitated benzoic acid was isolated and weighed.

RESULTS AND DISCUSSION

In dilute hydrochloric acid medium the rate of transformation of the acetyl derivative *I* does not depend on the proton concentration, which agrees with the findings[2,3] concerning the benzoyl derivative *II*. Ratio of the rate constants of the acetyl derivative *I* and benzoyl derivative *II* is 8 : 1 (Table II). Thiourea and acetic acid are the only reaction products.

TABLE I

Amount of the formed N-acetyl derivative *III* (mol %) from transformation of *I* into thiourea and *III* in phosphate and borax buffers

Buffer				*III* mol %
$[HPO_4^{-2}]$	$[H_2PO_4^-]$	$[HBO_2]$	$[NaBO_2]$	
$5 . 10^{-3}$	$5 . 10^{-3}$	—	—	53
$5 . 10^{-3}$	$2 . 10^{-3}$	—	—	65
—	—	$5 . 10^{-3}$	$2 . 10^{-3}$	96
—	—	$3 . 10^{-3}$	$2 . 10^{-3}$	87
—	—	$5 . 10^{-3}$	$10 . 10^{-3}$	62

In acetate buffers the rate constant increases (in all the cases) linearly with increasing concentration of acetate ion (the basic buffer component), being independent of acetic acid concentration in the buffer. All the experimental points k_{exp} lie at one straight line irrespective of pH of the buffer. The k_{exp} value extrapolated to zero buffer concentration agrees with that found in dilute hydrochloric acid (Fig. 1). Similar dependences were also found for the benzoyl derivative *II* (Fig. 2), the slope of the dependence k_{exp} *vs* acetate buffer concentration being, however, much smaller than that of the acetyl derivative *I*. Table II gives rate constants of the acetate-ion-catalyzed reactions of *I* and *II*. Again in phosphate buffers k_{exp} increases linearly with increasing basic buffer component, and for three different ratios of the buffer components the k_{exp} values lie at parallel straight lines (Figs 1 and 2). The intercepts at *y*-axis found by extrapolation to zero buffer concentration correspond to sum of rate constants of non-catalyzed hydrolysis (k_0) and hydroxyl--ion-catalyzed reaction (k_{OH}). Thus the observed rate constant k_{exp} is given by Eq. (*1*).

$$k_{exp} = k_0 + k_{OH} \cdot a_{OH} + k_B[B] , \qquad (1)$$

where [B] means the basic buffer component concentration.

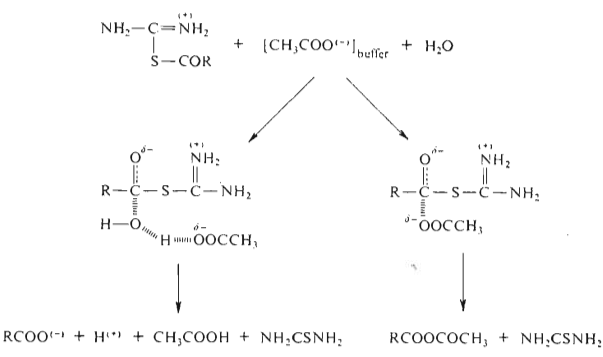
Rate constants of the reaction catalyzed by monohydrogenphosphate ion were evaluated similarly to those obtained with acetate (Table II). The k_{OH} rate constants were determined from the slope of dependence of the *y*-axis intercepts *vs* hydroxyl ion activity. The reaction catalyzed by the basic buffer component can be either

TABLE II

Values of rate constants of transformation of acetyl derivative *I* and benzoyl derivative *II* in aqueous buffer solutions at 25°C at ionic strength $I = 1$, k_0 (s^{-1}) rate constant of the non-catalyzed reaction. k_{OH} ($l\ mol^{-1}\ s^{-1}$) rate constant of the reaction catalyzed by hydroxyl ion, k_{AcO} ($l\ mol^{-1}\ s^{-1}$) rate constant of the reaction catalyzed by basic component of acetate buffer, k_{HPO_4} ($l\ mol^{-1}\ s^{-1}$) rate constant of the reaction catalyzed by basic component of phosphate buffer

Rate constant	Compound	
	I	*II*
$10^3 \cdot k_0$	5·8 ± 0·4	0·7 ± 0·05
$10^{-5} \cdot k_{OH}$	5·4 ± 1·2	2·1 ± 0·2
k_{AcO}	0·48 ± 0·05	0·086 ± 0·009
k_{HPO_4}	1·0 ± 0·1	0·1 ± 0·009

general base-catalyzed reaction or nucleophile-catalyzed one. Scheme 1 gives reaction mechanisms of the two types of catalysis in acetate buffer medium.



SCHEME 1

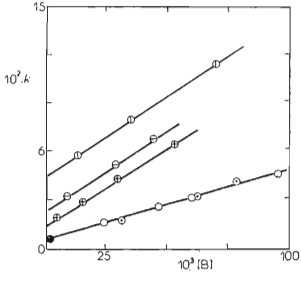


FIG. 1

Dependence of the observed rate constant k (s^{-1}) of decomposition of compound I on concentration of basic buffer component (B) at 25°C at ionic strength $I = 1$. ○ acetate, pH 4·70, ⊙ acetate, pH 5·10, ⊕ phosphate, pH 6·08, ⊖ phosphate. pH 6·46, ⦶ phosphate, pH 6·83, ● hydrochloric acid (10^{-3} mol l^{-1})

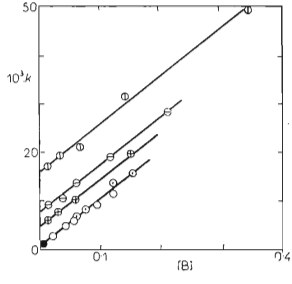


FIG. 2

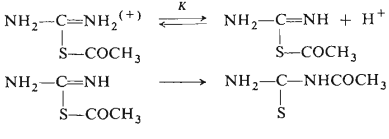
Dependence of the observed rate constant k (s^{-1}) of decomposition of compound II on concentration of basic buffer component (B) at 25°C at ionic strength $I = 1$. For description of experimental points see Fig. 1

From acetyl derivative *I* the nucleophilic catalysis produces acetanhydride which can be identified and quantitatively determined by reaction with aniline. A separate experiment of transformation of *I* in aqueous aniline (pH 5) revealed that acetanilide is formed quantitatively. Therefore, in the proper experimental series aniline was not added until after practically complete conversion of the S-acetyl derivative *I* into products (the shortest interval was 20 s). As the formed acetanhydride is hydrolyzed with water to acetic acid, a series of experiments were carried out in which aniline was added at gradually increasing intervals (20 s to 5 min). Amount of the formed acetanhydride was determined from the time dependence of acetanilide amount extrapolated to zero time (presuming the acetanhydride hydrolysis to be a pseudo-monomolecular reaction). As the conversion of acetyl derivative *I* under these conditions has a half-life below 5 s, and decomposition half-life of acetanhydride is about 5 min, the extrapolation to zero time is justified. The found value corresponds to 92% of theoretical amount of acetanhydride. Hence, general base catalysis (if any) makes at most 10%.

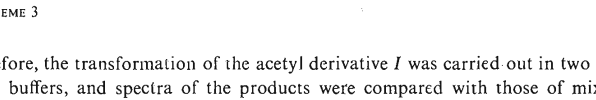
The same mechanism is presumed also in the case of the benzoyl derivative *II* which, on reaction with aniline, gives benzanilide in quantitative yields, too. The experiments could not be evaluated quantitatively, because under the reaction conditions used the rate of transformation of the benzoyl derivative and that of decomposition of the formed mixed anhydride were almost the same. Furthermore, a separate experiment with the mixed anhydride and aniline gave a mixture of acetanilide and benzanilide; hence quantitative evaluation would be too difficult.

Rate constant of the monohydrogenphosphate-ion-catalyzed reaction of the benzoyl derivative *II* is practically the same as that of the acetate-catalyzed reaction and in case of the acetyl derivative *I*, only twice as large (Table II), although monohydrogenphosphate is an about 100 times stronger base than acetate. This result is due to nucleophilicity of monohydrogenphosphate ion being (as compared with nucleophilicity of acetate) much lower than one would expect on the basis of its basicity. It is possible that in this case general base catalysis can be considerably significant, which was not proved by experiments.

The hydroxyl-ion-catalyzed reaction of the acetyl derivative *I* can proceed by two mechanisms, too. One mechanism is given in Scheme 2, the other consists in attack of carbonyl group of the protonated substrate *I* by hydroxyl ion to give acetate ion and thiourea (Scheme 3).



SCHEME 2

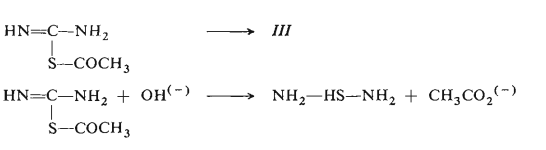


SCHEME 3

Therefore, the transformation of the acetyl derivative *I* was carried out in two phosphate buffers, and spectra of the products were compared with those of mixtures of thiourea and N-acetylthiurea (*III*). This comparison revealed that relative amount of N-acetylthiourea (*III*) in the reaction products corresponds to relative value of the expression $k_{OH} \cdot a_{OH}$ in the observed rate constant (Eq. (*3*)) found at the given buffer concentration and ratio. Hence the hydroxyl-ion-catalyzed reaction proceeds by Scheme 3.

In strongly basic media (pH > 10) elimination is the predominant reaction of the benzoyl derivative *II*, giving salt of thiobenzoic acid and urea[2,3]. Preparative decomposition of the acetyl derivative *I* in sodium hydroxide solution gave thiourea along with a small amount of urea, which means that elimination reaction is of very small significance in the case of the acetyl derivative *I*.

Under the reaction conditions given the neutral compound *I* can either rearrange to *III* or react with hydroxyl ion to give thiourea and acetate (Eqs (*A*) and (*B*)).



N-Acetylthiourea (*III*) is hydrolyzed under the reaction conditions with a half-life lower than 1 min (ref.[9]), hence the final products of reaction (*A*) are thiourea and acetate ion again. Though the reactions (*A*) and (*B*) are substantially different kinetically (rate of (*A*) being dependent on hydroxyl ion concentration whereas that of (*B*) being independent of it), the transformation is so fast in this case, that the kinetics cannot be measured. Therefore, experiments were carried out with low substrate concentrations in 0·01 and 0·1 mol l^{-1} sodium hydroxide in such a way that compound *I* was dissolved and, after about 7 s (when its transformation is practically finished), dihydrogenphosphate solution was injected in the mixture to make the final pH about 6. Compound *III* is practically stable under these conditions. Spectrum of the product indicates the presence of thiourea (its amount corresponding to quantitative transformation) and absence of *III*. Hence the predominant reaction consists in attack of carbonyl group of neutral compound *I* by hydroxyl ion.

Different behaviour of *I* and *II* is due to the fact that carbonyl group of acetyl is much faster attacked by hydroxyl ion (and by other nucleophiles, too) than is

carbonyl group of benzoyl. Also it is possible that the proper rearrangement of acetyl is slower than that of benzoyl, which cannot be proved, as it was impossible to measure pK_a value of the protonated *I*.To confirm the idea that increasing hydroxyl ion concentration favours the hydroxyl-ion-catalyzed hydrolysis of the neutral form to rearrangement of this form, we determined composition of reaction products in three mixtures boric acid–borate (Table I). Increasing pH value resulted in decrease of content of *III* (96%, 87%, 62%) and simultaneous increase of thiourea content. In these media the hydrolysis of *III* is so slow that it cannot affect results of the measurements.

Solvolysis of compound *I* has almost the same rate in methanol and in water. However, a small addition of water to methanol increased the solvolysis rate considerably. Therefore, we measured experimentally dependence of the rate constants of *I* and *II* on water concentration in methanol (Fig. 3). For the both compounds *I* and *II* the dependence has similar course. The maximum solvolysis rate is attained in methanol–water medium 75 : 25 (v/v) where it is about 3–4 times greater than the rate in water or neat methanol. In the region of the maximum rate (water–methanol 1 : 3) we analyzed the reaction product mixture obtained from the benzoyl derivative *II*. The found concentration ratio of benzoic acid to its ester (1 : 3·5) does not substantially differ from that of water to methanol in the analyzed reaction mixture. Hence the found considerable change in reaction rate caused by change of medium cannot be connected with different nucleophilicity of the two reacting components (*i.e.* water and methanol). Obviously the decisive factor is due to different ability of the medium to solvate the starting protonated substrate and the activated complex. Presuming the addition-elimination mechanism of the reaction, structure of the activated complex will resemble that of the tetrahedral intermediate. In the starting protonated substrate the charge is distributed on the nitrogen atoms, *i.e.* the sub-

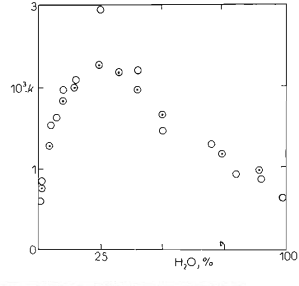
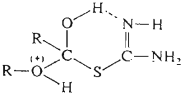


FIG. 3

Dependence of the observed rate constant k (s^{-1}) of solvolysis of compound *I* (○) and compound *II* (⊙) in 10^{-3} mol l^{-1} hydrochloric acid on water content (% by vol.) in methanol at 25°C

strate has a character of an ammonium salt. The tetrahedral intermediate has, most probably, the structure *IV* in which the charge of oxygen atom of the nucleophile is partially transferred to adjacent solvent molecules through hydrogen bonds.



IV

$R = CH_3$ or C_6H_5
$R' = CH_3$ or H

Thus this case represents a certain analogy to transfer of positive charge from ammonium ion to solvent molecules during its dissociation (*C*).

$$R{-}NH_3^{(+)} + H_2O \rightleftarrows H_3O^{(+)} + R{-}NH_2 \qquad (C)$$

Dependence of dissociation constant of protonated amines on composition of aqueous-methanolic medium has a similar course with maximum values about the ratio methanol to water 3 : 1. In this mixture the dissociation constants are greater than those in water by about one order of magnitude[10]. Formation of the activated complex of a solvolytic reaction does not involve such a marked change of charge transfer as that of dissociation of an ammonium ion, and, therefore, a change of medium does not affect the solvolysis rates so markedly.

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