Kinetics and Mechanisms of Conversion of Methyl 1-(Butylcarbamoyl)-2-benzimidazolecarbamate (Benomyl) to Methyl 2-Benzimidazolecarbamate (MBC)

Jean-Pierre Calmon* and Daniel R. Sayag

The investigation of the effect of pH on the kinetics of hydrolysis of benomyl in acidic media, together with a search for buffer catalysis and hydrogen–deuterium isotope effect, do not preclude totally general base catalysis; however, its role is not very important and therefore the involvement of the solvent in the reaction mechanism can be neglected. On the contrary, the study of the ionization of benomyl in acidic media, the good agreement between the pK_a values determined both spectrophotometrically and kinetically, as well as the positive value of the entropy of activation, provide decisive arguments for a mechanism which proceeds by spontaneous intramolecular catalysis operating on the neutral form of the substrate.

Methyl 1-(butylcarbamoyl)-2-benzimidazolecarbamate (benomyl) is the active ingredient in Du Pont Benlate systemic fungicide which is being extensively used for disease control in many crops throughout the world; its fungitoxic activity was first reported by Delp and Klopping in 1968. Benomyl is unstable in aqueous media and is rapidly converted to methyl 2-benzimidazolecarbamate (MBC) by removal of the butylcarbamoyl side chain (Clemons and Sisler, 1969). Within plants, benomyl is translocated with the upward moving sapstream and rapidly breaks down to MBC (Peterson and Edgington, 1970), so that benomyl itself is seldom detected in the sap. Sunlight, heat, and various solvents enhance this conversion (Kilgore and White, 1970; Chiba and Doornbos, 1974). In alkaline media, the hydrolysis of benomyl yields other derivatives, namely a triazinobenzimidazole (STB) and a benzimidazolylbutylurea (BBU) (White et al., 1973). However, MBC practically constitutes a major component of the local residue on plants for extended periods of time, even under alkaline conditions (Baude et al., 1973).

The investigations carried out on benomyl to date essentially had an analytical character. The hydrolysis of benomyl was considered to be fast until intact benomyl could be detected on certain plants several weeks after application (Jhooty and Singh, 1972; Brown and Albrigo, 1972), which showed that benomyl breakdown could be slower than had been thought previously. It was therefore interesting, from a theoretical as well as from a practical standpoint, to investigate the mechanisms of the transformations of benomyl and its metabolites.

The kinetic study we carried out allowed us to elucidate the effect of pH and that of various solvents on the rate of conversion of benomyl and to gain some insight about the reaction mechanisms involved in the different pH ranges. This paper deals with the conversion of benomyl to MBC in acidic and neutral media.

EXPERIMENTAL SECTION

Apparatus. A Unicam Model SP 800 recording spectrophotometer, equipped with a thermostated multiple cell compartment, was used for all spectroscopic measurements. The pH measurements were carried out using a Metrohm E 300 B pH meter.

Chemicals. All chemicals used were of analytical or reagent grade. Buffers were made from deionized water distilled from alkaline potassium permanganate and from analytical grade materials used without further purification. Aqueous solutions for various pH ranges were prepared using hydrochloric acid, acetic acid–sodium hydroxide, and potassium dihydrogen phosphate–sodium hydroxide.

Benomyl was provided by Du Pont de Nemours-France. MBC was obtained through hydrolysis of benomyl; its chemical characteristics were identical with those reported in the literature.

Uv Spectra. Water–methanol solutions of benomyl and MBC exhibited the following absorptions [λ_{max} nm (log ϵ); s = shoulder]: benomyl, protonated species, 230 (4.1), 276 (4.1), 283 (4.1); neutral species, 223 (4.3), 240 (4.0), 255 (3.9), 263 (3.9) s, 285 (4.2) s, 294 (4.3); MBC, protonated species, 225 (4.2), 275 (4.2) s, 282 (4.2); neutral species, 241 (4.0), 281 (4.1) s, 287 (4.2), 294 (3.8) s.

Benomyl and MBC uv spectra look very much like those of benzimidazole which exhibits two groups of bands near 245 nm (y) and 280 nm (x). The electron withdrawing groups of benomyl (1-butylcarbamoyl and 2-methylcarbamate) and MBC (2-methylcarbamate) alter the uv spectrum of the benzimidazole ring; an additional band (sharper for benomyl) appears near 294 nm and the band at 245 nm shifts towards shorter wavelengths.

Protonation of the neutral molecule of the derivatives investigated results in a hypsochromic displacement of the bands of the benzimidazole ring. The intensity of the x band is increased but its shape is not changed; the y band is attenuated. This hypsochromic shift corresponds to an increase in electronegativity of the nitrogen atoms of the benzimidazole ring.

Kinetic Measurements. All reactions were carried out at 25 ± 0.1°C (unless otherwise specified) in tightly stoppered 1-cm quartz cells containing the appropriate buffers in both sample and reference compartments. Because of the low water solubility of benomyl, the kinetics of hydrolysis of benomyl were studied in 1:1 (v/v) water–methanol, the ionic strength being maintained constant at 1.0 M by the addition of potassium chloride. The pH values quoted for 1:1 water–methanol solutions are the measured values without further correction. The change in optical density of the substrate was followed at suitable wavelengths. Initial repetitive scans of the uv region established that these reactions held tight isosbestic points, indicating the absence of intermediates.

The absorbance vs. time plots gave the pseudo-first-

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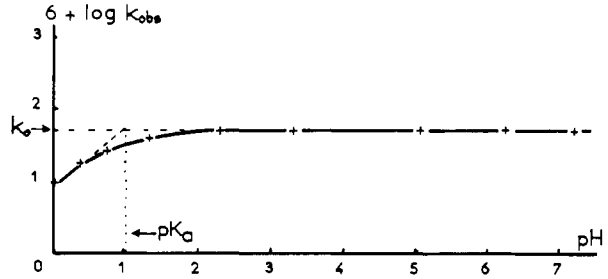


Figure 1. Plot of the logarithms of the observed rate constants vs. pH for the hydrolysis of benomyl to MBC at 25°C in water-methanol with μ = 1.0 (with KCl): $\lambda_M \simeq$ 294 nm; $c = 5 \times 10^{-5}$ mol/l.

order rate constants graphically, using the experimental infinity value. The observed rate constants k_{obsd} were obtained by plotting log $(A_t - A_\infty)$ vs. time, where A_∞ and A_t are the absorbance readings at infinity and at time t, respectively: $\log (A_t - A_\infty) = \log A_0 - (k_{obsd}/2.303)t$.

pK_a Measurements. The ionization constant of benomyl was determined spectrophotometrically, taking advantage of the differences in the absorbances of the protonated and neutral forms at 294 nm. The plot of optical density vs. pH was a sigmoid, the inflection point of which gave the pK_a.

Thermodynamic Parameters of Activation. When the logarithms of the observed pseudo-first-order rate constants k_{obsd} were plotted vs. $1/T$, straight lines were observed, the slopes of which multiplied by $-2.303R$ gave the Arrhenius activation energy E_a. The enthalpies and entropies of activation $\Delta H^\ddagger$ and $\Delta S^\ddagger$ were respectively obtained from the following equations.

$$E_a = \Delta H^\ddagger + RT$$

$$\log k_{obsd} = \log (eK/h) + \log T - E_a/2.3RT + \Delta S^\ddagger/2.3R$$

RESULTS

Kinetic Data. Plots of the logarithms of the observed pseudo-first-order rate constants k_{obsd} against pH for benomyl hydrolysis are shown in Figure 1. This pH–rate profile shows that in strongly acidic media (pH < 2.5) the hydrolysis is inhibited by hydronium ions whereas over the pH range 2.5–7.0, the reaction rate is pH independent.

A detailed investigation of the uv spectra recorded at the end of reaction shows that over the whole pH range investigated the final product is methyl 2-benzimidazolecarbamate (MBC).

The plateau in the pH–rate profile, i.e. an extended range where the reaction velocity is pH independent and therefore independent from the composition of the reaction medium, suggests either involvement of the solvent by nucleophilic attack of water, or spontaneous intramolecular catalysis. To distinguish between these two possibilities, additional experimental data are needed so as to know whether the buffer bases can be involved in the reaction mechanism by proton abstraction from the substrate, as the solvent can do.

Effect of Buffer Concentration. The effect of buffer concentration on the reaction rate was studied for acetic acid–sodium acetate mixtures; the observed rate constant increased with buffer concentration (Figure 2). The catalysis is dependent only on the free base component of the buffer; this is best seen in terms of a plot of $(k_{obsd} - k_0)/([CH_3COOH] + [CH_3COO^-])$ against $[CH_3COO^-]/([CH_3COOH] + [CH_3COO^-])$, which gives a straight line (Figure 3). For a buffer base molar fraction equal to 1.0, the intercept differences provide the value of the catalytic

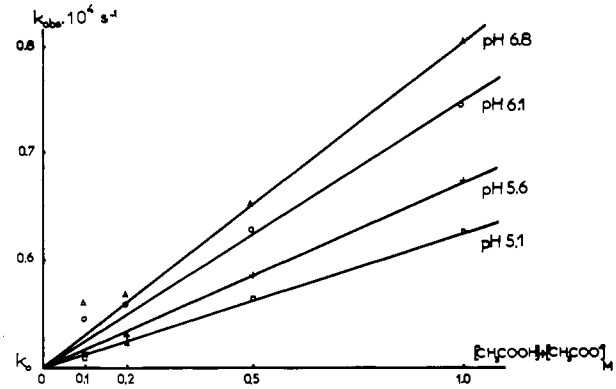


Figure 2. Plot of the observed rate constants vs. buffer concentration for the hydrolysis of benomyl to MBC at 25° C in water-methanol with μ = 1.0 (with KCl). k_0 is the observed plateau rate; $k_0 = 0.5 \times 10^{-4}$ s^{-1}.

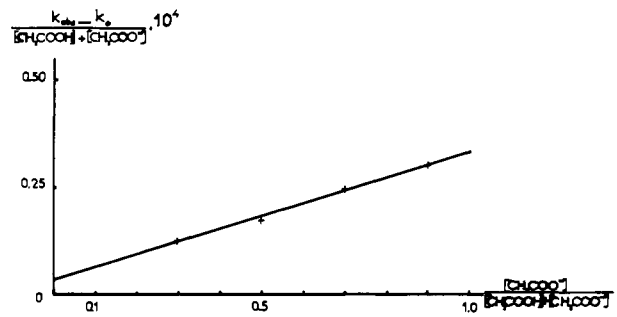


Figure 3. Plot of the observed rate constants against the free base concentration of the buffer for the hydrolysis of benomyl to MBC at 25° C in water-methanol with μ = 1.0 (with KCl).

constant for the acetate ion, $k_B^{CH_3COO^-}$, which is represented by $(k_{obsd} - k_0)/([CH_3COOH] + [CH_3COO^-]) \simeq 0.3 \times 10^{-4}$ M^{-1} l. s^{-1}.

Likewise, the effect of the formic acid–sodium formate buffer concentration on the hydrolysis rate results in a catalytic constant for the formate ion, $k_B^{HCOO^-} \simeq 0.2 \times 10^{-4}$ M^{-1} l. s^{-1}.

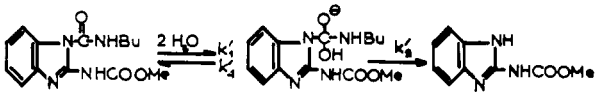
The values of these catalytic constants are much lower than those reported in literature for general base catalysis. Therefore, general base catalysis, if present, is anomalously weak. However, these catalytic constants are of the same order of magnitude as k_0, the observed plateau rate. Hence the possibility of a weak involvement of general base catalysis cannot be entirely ruled out.

Deuterium Oxide Solvent Isotope Effect. As the search made for buffer catalysis was not conclusive, the investigation of the hydrogen–deuterium isotope effect on the hydrolysis kinetics was considered so as to know whether the rate-determining step involved a proton transfer, in which case the reaction would be markedly slowed down in deuterium oxide. The kinetic study carried out in heavy water at pH 3.0 led to an isotopic effect, k_H/k_D = 1.3. Such a value is not consistent with a rate-limiting proton transfer; therefore, the occurrence of general base catalysis can be ruled out. The most likely explanation for this low value is a secondary isotopic effect.

Protonation of Benomyl. In the pH range below 2.5, where the reaction rate is decreasing as the acidity of the medium is increasing, a protonated form of benomyl was brought out. The ionization pK_a was determined spectrophotometrically under the same experimental conditions as those of the kinetic study: pK_a = 1.1.

Thermodynamic Functions of Activation. The rate constants measured at 10 and 25°C were used to compute

Scheme I



the enthalpy and entropy of activation corresponding to the plateau of the pH–rate profile: $\Delta H^{\ddagger} = +25.4$ kcal; $\Delta S^{\ddagger} = +7.3$ cal deg^{-1} mol^{-1}.

DISCUSSION

The results presented in the previous section can be rationalized in terms of two possible mechanistic schemes for the reaction.

Hydrolysis Via a Tetrahedral Intermediate Transition State. The reaction of the solvent on the substrate involves two water molecules: the first one acts as a nucleophilic agent by attacking the carbonyl group of the butylcarbamoyl chain of the substrate, with formation of an anion; the other one behaves like a base catalyst by abstracting a proton (Scheme I).

The hydrolyses of benomyl were characterized by tight isosbestic points, showing that the rate of decomposition of the reactants is equal to the rate of formation of the products. If S stands for the substrate and A for the anion, the steady-state approximation applied to the tetrahedral intermediate leads to the following equations (1–4).

$$v = -(\mathrm{d}[S]/\mathrm{d}t) = k_{obsd}[S] = k_2'[A] \quad (1)$$

$$k_1'[S] = k_{-1}'[A][H_3O^+] + k_2'[A] \quad (2)$$

whence

$$A = \frac{k_1'[S]}{k_{-1}'a_H + k_2'} \quad (3)$$

and

$$k_{obsd} = \frac{k_1'k_2'}{k_{-1}'a_H + k_2'} \quad (4)$$

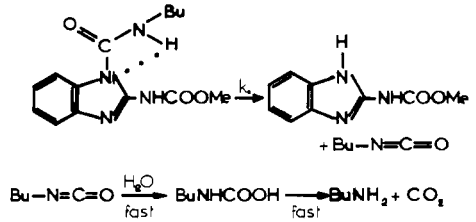
This rate law is in agreement with the pH–rate profile. It accounts for inhibition by hydronium ions when a_H values are sufficiently high, whereas, in less acidic media, corresponding to the plateau of the pH profile, the $k_{-1}'a_H$ term becomes negligible with respect to k_2' and $k_{obsd} \simeq k_1' = k_0$. The first step is then rate determining. On the basis of the proposed mechanism, the reaction should be submitted to general base catalysis; the absence of important buffer effects is not consistent with the above mechanism. Besides, this mechanism does not allow an explanation of the solvolysis observed in various solvents (Kilgore and White, 1970; Chiba and Doornbos, 1974).

As several features argue against this reaction mechanism, it has to be discarded, though such a reaction pathway is known for the hydrolysis of *N*-acylimidazoles and *N*-benzoylimidazoles (Choi and Thornton, 1974).

Hydrolysis Via Intramolecular Catalysis. The conversion of the substrate is due to a spontaneous intramolecular catalysis; the formation of an H bond between the hydrogen of the nitrogen of the butylcarbamoyl side chain and the lone pair of the nitrogen atom N_1 of the benzimidazole ring leads to a four-membered ring, which, being unstable, opens and yields methyl 2-benzimidazolecarbamate and butyl isocyanate. This isocyanate intermediate reacts rapidly with water to form the corresponding carbamic acid which decomposes almost immediately to CO_2 and butylamine (Scheme II).

Such a mechanism was suggested for the hydrolysis of urea in acidic media (Show and Walker, 1956) and also accounts for that of phenylurea (Calmon and Doux, 1973).

Scheme II



Furthermore, whereas extemely slow rates are encountered for the hydrolysis of urea, it is noteworthy that, by the incorporation of one nitrogen atom of a ureido function into a heterocyclic system, the rates of hydrolysis are increased greatly.

The reaction inhibition observed in the most acidic solutions can be explained by the protonation of the benomyl molecule. By analogy with benzimidazole, we assume that the protonation takes place at the ring nitrogen atom N_3 (unsaturated), which leads, by electronic effect, to a decrease in the electronegativity of the ring nitrogen atom N_1, and therefore to the inhibition of the intramolecular catalysis. The reactive species is the nonprotonated form of the substrate. This reaction scheme can be written as follows:

$$S + H_3O^+ \overset{1/K_a}{\rightleftharpoons} SH^+ + H_2O$$

$$S \overset{k_0}{\rightarrow} BCM$$

where K_a is the dissociation constant of the protonated substrate. The rate law is expressed as:

$$k_{obsd}([SH^+] + [S]) = k_0[S] \quad (5)$$

whence

$$k_{obsd} = k_0K_a/(K_a + a_H) \quad (6)$$

This rate law, which cannot be kinetically distinguished from that suggested for the reaction pathway involving a tetrahedral intermediate, is consistent with the experimental data: when $a_H \ll K_a$ (pH >2.5), the observed rate constant becomes equal to k_0; $k_{obsd} \simeq k_0 = 0.5 \times 10^{-4}$ s^{-1} (k_0 is therefore the spontaneous rate constant); when $a_H \gg K_a$ (pH ≪2.5), k_{obsd} is proportional to $1/a_H$; the pH–rate profile (Figure 1) becomes a straight line of slope near unity, which can be explained by the protonation of the substrate. This part of the pH profile allows an estimation of the pK_a of the protonated form of the substrate at the intersecting point of the straight line of slope unity and of the plateau observed between pH 2.5 and 7.0 (see Figure 1). The pK_a value obtained kinetically (p$K_a \simeq 1.0$) is in good agreement with that of 1.1, determined spectrophotometrically. This important result does support the inhibition of the reaction by the protonated form of the substrate, according to the proposed mechanism which involves an intramolecular catalysis.

The positive value of the entropy of activation adds further support to the spontaneous intramolecular catalysis as it indicates an increase in the degrees of freedom of the system. On the contrary, in the reaction scheme involving a tetrahedral intermediate, a negative value should be expected for the entropy of activation, because such a pathway implies a loss of degrees of freedom.

CONCLUSIONS

This work, besides its theoretical interest, allows a better understanding of the environmental behavior of benomyl. The conversion of benomyl to MBC, which takes place in the sapstream within the plant, is due to spontaneous

intramolecular catalysis, the involvement of enzymatic systems not being necessary. The usual conditions of treatment with benomyl (foliar sprays) correspond to pH values near neutrality and lead to its conversion to MBC via intramolecular catalysis. The conversion time within the plant seems to be of the same order of magnitude as that observed in aqueous media, neutral or slightly acidic (Peterson and Edgington, 1970); however, the concentrations used can increase the persistence of the fungicide (employed as a suspension since it is water insoluble) which will be made available only gradually.

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Received for review July 23, 1975. Accepted November 11, 1975.

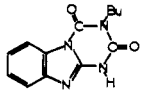
Kinetics and Mechanisms of Conversion of Methyl 1-(Butylcarbamoyl)-2-benzimidazolecarbamate (Benomyl) to 3-Butyl-2,4-dioxo[1,2-*a*]-*s*-triazinobenzimidazole (STB) and 1-(2-Benzimidazolyl)-3-*n*-butylurea (BBU)

Jean-Pierre Calmon* and Daniel R. Sayag

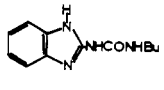
The investigation of the effect of pH on the kinetics of hydrolysis of benomyl in alkaline media introduced several reaction mechanisms. In mildly alkaline media (pH <12), the study of the ionization of benomyl showed that its conversion to STB proceeds by an E1cB elimination mechanism, followed by a fast cyclization; the reactive species is the anionic form of the substrate which results from proton abstraction on the nitrogen of the methylcarbamate group. The pK_a'' values determined both spectrophotometrically and kinetically are in good agreement. In strongly alkaline media (pH >12), benomyl is converted into STB via a dianion. The conversion of STB to BBU which occurs only in very strongly alkaline media (pH >13.5) is first order with respect to hydroxide ion.

White et al. (1973) showed that, in alkaline media, methyl 1-(butylcarbamoyl)-2-benzimidazolecarbamate (benomyl) is converted into 3-butyl-2,4-dioxo[1,2-*a*]-*s*-triazinobenzimidazole (STB). STB also is a systemic fungicide (Bose and White, 1973). This reaction is fast and quantitative.

The same authors also observed, in standing alkaline solutions of STB, the precipitation of another derivative, whose amount increased with time, temperature, and alkalinity: this compound, which results from the opening of the triazine ring of STB, was isolated and characterized as being 1-(2-benzimidazolyl)-3-*n*-butylurea (BBU).



STB



BBU

The present paper is concerned with the effect of pH on the rate of conversion of benomyl in order to elucidate the reaction mechanisms which are involved in different pH ranges in alkaline media.

EXPERIMENTAL SECTION

Apparatus. A Unicam SP 800 recording spectrophotometer, equipped with a thermostated multiple cell compartment, was used for all spectroscopic measurements. The pH measurements were carried out using a Metrohm E300B pH meter.

Chemicals. All chemicals used were of analytical or reagent grade. Aqueous solutions for various pH ranges were prepared using potassium dihydrogen phosphate–sodium hydroxide, boric acid–sodium hydroxide, tris(hydroxymethyl)aminomethane–hydrochloric acid, disodium hydrogen phosphate–sodium hydroxide, and sodium hydroxide.

Benomyl was provided by Du Pont de Nemours-France. STB and BBU samples were a gift of E. R. White. MBC was obtained through hydrolysis of benomyl; its chemical characteristics were identical with those previously reported.

Uv Spectra. Water–methanol solutions of benomyl, MBC, STB, and BBU exhibited the following absorptions [λ_{max} nm (log ϵ); s = shoulder]: benomyl, neutral species, 223 (4.3), 240 (4.0) s, 255 (3.9), 263 (3.9) s; 285 (4.2) s, 294 (4.3); anionic species, 243 (4.1), 293 (4.2); MBC, neutral species, 241 (4.0), 281 (4.1) s, 287 (4.2), 294 (3.8) s; anionic species, 252 (4.0), 294 (4.2), 300 (4.2) s; STB, anionic species, 275 (4.1), 285 (4.1) s; BBU, 293 (4.2).

Benomyl and MBC uv spectra look very much like those of benzimidazole which exhibits two groups of bands near 245 nm (*y*) and 280 nm (*x*). Deprotonation of the neutral molecule of these derivatives results in a decrease in electronegativity of the nitrogen atoms of the benzimidazole ring; resonance is enhanced and the bands *x* and *y* undergo a bathochromic displacement and become

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