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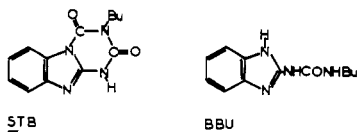
The present paper is concerned with the effect of pH on the rate of conversion of benomyl in order to elucidate the reaction mechanisms which are involved in different pH ranges in alkaline media.

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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Table I. Observed Rate Constants for Hydrolysis of Benomyl in Na_2HPO_4-NaOH Buffers at 25°C in Water-Methanol (μ = 1.0, KCl)

$[Na_2HPO_4]$, M	0.1	0.05	0.02	0.01	0.1	0.05	0.02	0.01
pH	10.8	10.75	10.6	10.25	11.7	11.65	11.45	11.25
$k_{obsd} \times 10^{-4}$, s^{-1}	8.35	8.06	8.16	8.16	8.35	8.16	8.45	8.35

merged under the same envelope, the shift being more important for band y than for band x.

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 the reactions held tight isosbestic points, indicating the absence of intermediates.

The absorbance vs. time plots gave the pseudo-first-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 - $(k_{obsd}/2.303)t$. When STB is converted to BBU, the absorbance is increasing and the observed rate constants are obtained from log $(A_\infty - A_t)$ = log A_∞ - $(k_{obsd}/2.303)t$.

pK_a Measurements. The ionization constant of benomyl was determined spectrophotometrically taking advantage of the differences in the absorbance of the deprotonated and neutral forms at 300 nm. The optical density vs. pH plot was a sigmoid, the inflection point of which gave the pK_a value.

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.

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

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

I. REACTION OF CONVERSION OF BENOMYL TO STB RESULTS

Kinetic Data. Figure 1 shows the plot of the logarithms of the observed pseudo-first-order rate constants k_{obsd} against pH for benomyl hydrolysis. This pH–rate profile exhibits an inflection which seems to reflect a change in the degree of ionization of benomyl as a function of pH and which points to a changeover in reaction mechanism with respect to acidic and neutral media. The rate constant is pH independent between pH 10.5 and 12. Above pH 12, the observed pseudo-first-order rate constants are proportional to the hydroxide ion concentration as shown by the linearity of a plot of k_{obsd} against $[OH^-]$; Figure 2. The reaction rate can then be expressed as:

$$k_{obsd} = k_1 + k_2[OH^-] \qquad (1)$$

with k_1 = 8.2 × 10^{-4} s^{-1} and k_2 = 3 × 10^{-3} M^{-1} l. s^{-1}.

Effect of Buffer Concentration. The effect of varying buffer concentration on the reaction rate was investigated at pH 9.2 in borax and at pH 10.8 and 11.7 in disodium
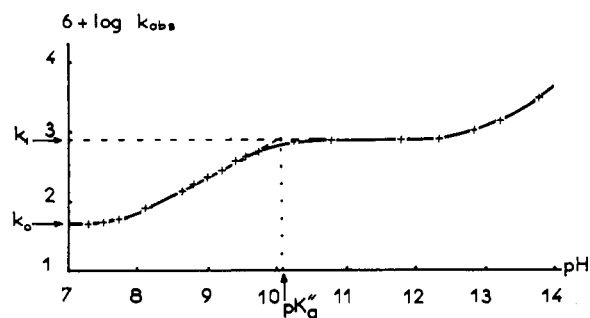

Figure 1. Plot of the logarithms of the observed rate constants vs. pH for the hydrolysis of benomyl to STB at 25°C in water-methanol (μ = 1.0, KCl); $\lambda_M \simeq 294$ nm; $c = 5 \times 10^{-5}$ mol/l.
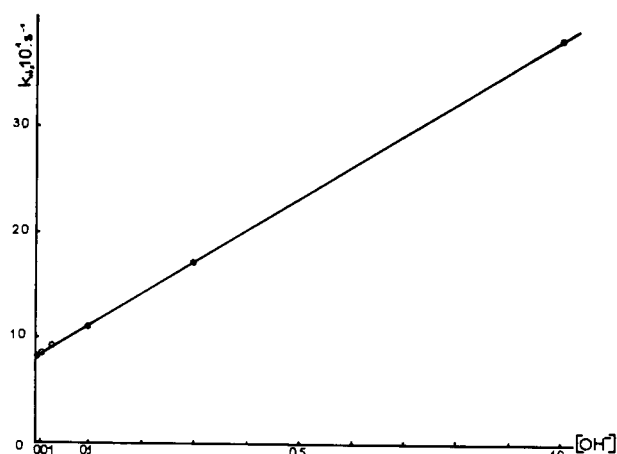

Figure 2. Plot of the observed rate constants vs. hydroxide ion concentration for the hydrolysis of benomyl to STB at 25°C in water-methanol (μ = 1.0, KCl).

hydrogen phosphate–sodium hydroxide buffers (Table I). The observed rate constant was invariant with dilution, which means that the base components of the buffer mixtures have no effect on the reaction rate.

Ionization of Benomyl. Spectral comparisons point to the existence of a deprotonated form of benomyl. The pK_a of ionization was determined spectrophotometrically in the same experimental conditions as those of the kinetic study: $pK_a'' = 10.15$.

Uv Spectra. A detailed investigation of the spectra recorded at the end of reaction between pH 10 and 14 shows that the final product is STB. However, between pH 7 and 10, a gradually decreasing participation of MBC is observed in the spectra recorded.

Deuterium Oxide Solvent Isotope Effect. 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 rate would be markedly changed in deuterium oxide. The kinetic study carried out in heavy water led to an isotopic effect $k_{H_2O}/k_{D_2O} \simeq 1$ for $[OD^-]$ = 2 × 10^{-3}, which indicates, as well as lack of buffer catalysis, that the reaction proceeds through preequilibrium ionization of benomyl.

Thermodynamic Functions of Activation. The rate constants measured at 10 and 25°C allowed calculation of the values of the enthalpy and entropy of activation

Scheme I
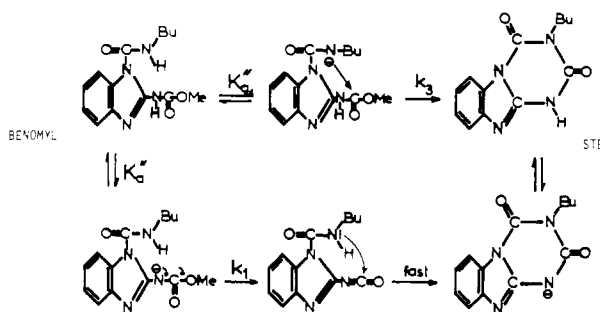
Scheme II

corresponding to the plateau of the pH–rate profile: $\Delta H^{\ddagger}$ = +18.1 kcal; $\Delta S^{\ddagger}$ = –12 cal deg^{-1} mol^{-1}.

DISCUSSION

Mildly Alkaline Media (pH <12). Two possible mechanisms of hydrolysis which are consistent with this kinetic behavior are outlined in Scheme I. In both cases, the reactive species is the conjugate base of the substrate, but proton abstraction can theoretically occur at the nitrogen atom of either the butylcarbamoyl group or the methylcarbamate group.

The presence of isosbestic points on the uv spectra illustrates that the rate of reactant decomposition is equal to the rate of product formation. These two reaction pathways lead to two rate laws of the same kind:

$$k_{obsd}''' = k_3 K_{a1}''/(K_{a1}'' + a_H) \qquad (2)$$

$$k_{obsd}'' = k_1 K_a''/(K_a'' + a_H) \qquad (3)$$

These two equations are kinetically equivalent at all pH values and cannot be used to distinguish the two pathways. These two rate laws are in agreement with the experimental data (Figure 1): when $a_H \ll K_a''$, k_{obsd} is pH independent; the pH–rate profile exhibits a plateau; when $a_H \gg K_a''$, k_{obsd} is proportional to $1/a_H$; then the pH profile becomes a straight line of slope unity.

In order to distinguish between these two reaction mechanisms, we defined the position of the most mobile proton. When heavy water is introduced in a deuteriochloroform solution of benomyl, the NH proton signal of the methylcarbamate group (δ = +11.4 ppm) vanishes on NMR spectra. Furthermore, the NH proton signal of the butylcarbamoyl group appears as a broad triplet (Keith and Alford, 1970), which bears out that it is not very mobile.

Therefore, in mildly alkaline media, the conversion mechanism of benomyl to STB seems to be an E1cB elimination, followed by a fast cyclization. E1cB reactions are elimination reactions proceeding by two steps: proton abstraction is followed by unimolecular elimination from the conjugate base of the substrate, elimination which results from the stabilization of the anion by intramolecular nucleophilic attack (Banthorpe, 1963). The rate-determining step is elimination of methoxide ion from the anion (k_1) to give the isocyanate intermediate which is very reactive.

Deuterium oxide solvent isotope effects can be used as evidence for E1cB mechanisms when the ionization of the substrate lies within the range of hydroxide ion concentration used: for the plateau rate, k_{H_2O}/k_{D_2O} = 1.0 (Tobias and Kezdy, 1969). Results for benomyl ($k_{H_2O}/k_{D_2O} \simeq 1$) are therefore confirmatory evidence for an E1cB mechanism.

The negative value of the entropy of activation cannot be used in support of an E1cB mechanism although it could be expected since methoxide ion is a poor leaving group
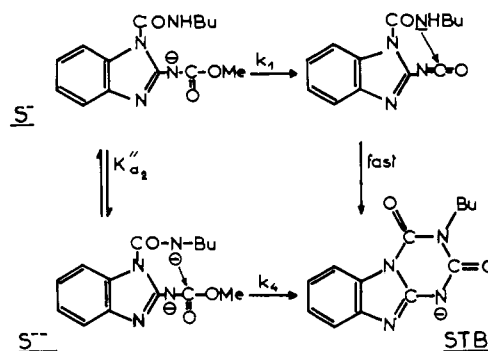
(Christenson, 1964) and because of the bulkiness of the transition state.

The pH–rate profile (Figure 1) allows a kinetic determination of the pK_a'' relative to proton abstraction from the substrate: the intersecting point of the straight line preceding the plateau (8 < pH < 9.5) and of the plateau (10.5 < pH < 12) provides a value of 10.15. The pK_a'' value thus obtained kinetically is in excellent agreement with that determined spectrophotometrically.

However, the slope of the straight line (8 < pH < 9.5) preceding the plateau of the pH profile is different from unity, whereas a linear dependence of log k_{obsd} on pH of slope +1 should be expected. The most likely explanation is that there is a changeover in mechanism in that pH range; when pH < pK_a'', spontaneous intramolecular catalysis (previously described in acidic and neutral media) is competing with E1cB elimination. A detailed investigation of the uv spectra recorded at the end of reaction bears out this interpretation: the spectra, which are complex, can be assigned to MBC–STB mixtures. As pH increases further and is approaching pK_a'', the E1cB mechanism preponderates and STB becomes the predominant final product.

Strongly Alkaline Media (pH >12). In strongly alkaline media, the pH–rate profile is curving upward (Figure 1), which may indicate a change in the degree of ionization of the substrate and characterizes a further changeover in reaction mechanism. This can be interpreted as follows: a second deprotonation, occurring at the nitrogen of the butylcarbamoyl group, yields a dianion which, by intramolecular nucleophilic attack of that nitrogen on the carbonyl of the methylcarbamate group, is converted into STB (Scheme II).

Therefore, between pH 12 and 14, there is a competition between two different reaction mechanisms involving two reactive species: a monoanion, S$^-$, and a dianion, S^{2-}. The reaction rate can then be expressed as:

$$V = k_{obsd}([S^-] + [S^{2-}]) = k_1[S^-] + k_4[S^{2-}] \qquad (4)$$

which, on introducing the rapid preequilibrium constant K_{a2}'', leads to:

$$k_{obsd} = \frac{k_1 K_w + k_4 K_{a2}''[OH^-]}{K_w + K_{a2}''[OH^-]} \qquad (5)$$

The order of magnitude of K_{a2}'' can be estimated from the shape of the pH–rate profile: 15 < pK_{a2}'' < 16. Therefore, between pH 12 and 14, $K_{a2}''[OH^-] \ll K_w$ and $k_{obsd} \simeq k_1$ + $k_4(K_{a2}''/K_w)[OH^-]$ which is consistent with the experimental data previously mentioned (see eq 1).

II. REACTION OF CONVERSION OF STB TO BBU

In very strongly alkaline media ([OH$^-$] > 1.0), after the fast conversion of benomyl to STB, a much slower reaction develops. The slope of the plot of log k_{obsd} against log
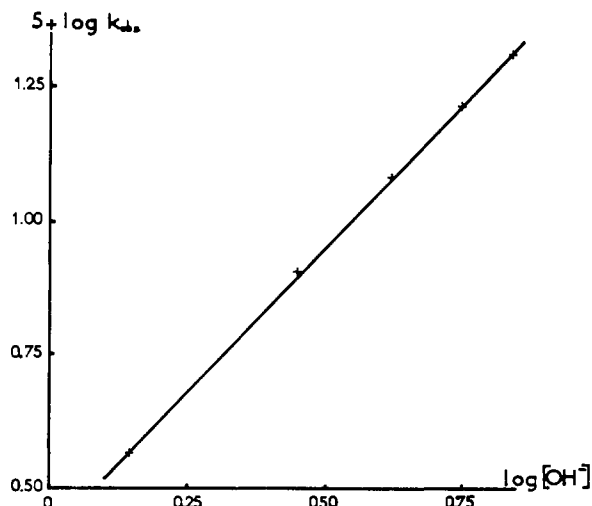
Figure 3. Plot of the logarithms of the observed rate constants vs. the logarithms of hydroxide ion concentration for the hydrolysis of STB to BBU at 25°C in water-methanol.

[OH-] is essentially 1.0 (Figure 3), indicating that the reaction has a simple first-order dependence on hydroxide ion concentration. The opening of the triazine ring of STB results in BBU formation in the reaction medium. This interpretation is consistent with the observations of White et al. (1973). The nucleophilic attack of hydroxide ion at the carbonyl next to the nitrogen atom N_1 of the benzimidazole ring causes the opening of the triazine ring of STB. This reaction is much slower than the cyclization of benomyl to STB: when [OH-] = 1.0, k_{obsd} = 0.2 × 10^{-4} s^{-1}; [OH-] = 7.0, k_{obsd} = 2 × 10^{-4} s^{-1}.

CONCLUSIONS

These results are of theoretical as well as of practical value and allow a better understanding of the chemical fate of benomyl in various conditions.

Benomyl can, in some circumstances, be submitted to alkaline conditions. Some anticryptogamic agents, such as the Bordeaux mixture, are alkaline. Local alkaline conditions can prevail in soils after recent or excessive liming. Heat treatments and alkaline peeling solutions are often used in fruit processing. In these various cases, the involvement of the reaction pathways leading to STB and BBU is quite possible. The environmental impact of either STB or BBU is not well known to date. However, it seems that, in normal practical use situations, the residue levels of benomyl or its derivatives are very low. As for the aerial parts of plants, even after alkaline treatments, intact benomyl constitutes the major component of the local residue on the leaves (Baude et al., 1973); the fast drying-out of the spray droplets prevents noticeable further degradation of benomyl.

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Specificity of the Vanillin Test for Flavanols

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The reaction with vanillin in acidic solution was previously considered to be a specific test for flavanols. In this work the specificity of the vanillin reaction was reexamined by testing the reactivity of 15 flavonoid and two chromone compounds. In addition to flavanols, the dihydrochalcones phloretin and phloridzin gave significant color development. Flavanone and flavanonol aglycones reacted weakly. The structural requirements for a positive reaction have been deduced. The vanillin–HCl screening test, used by plant breeders, has been modified to prevent the possible occurrence of a false positive interpretation due to the presence of anthocyanins in plant materials. The anthocyanidins cyanidin, pelargonidin, and peonidin were identified in alfalfa (Medicago sativa L.) herbage, and several other herbaceous legumes were examined for the presence of anthocyanins. The possibility of interference by dihydrochalcones or anthocyanins should be considered when the vanillin–HCl reaction is used for the detection and quantitative analysis of flavanols in plant materials.

Alfalfa (Medicago sativa L.), red clover (Trifolium pratense L.), and white clover (T. repens L.) are legume forages which may cause ruminant bloat when they are grazed by cattle or sheep, but sainfoin (Onybrychis vi-

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ciaefolia Scop.) and birdsfoot trefoil (Lotus corniculatus L.) are legume forages which do not cause bloat. The observation that protein precipitants are present in sainfoin and birdsfoot trefoil herbage, but absent from alfalfa, red clover, and white clover herbage, has led to the conclusion that protein precipitants are responsible for the nonbloating property of sainfoin and trefoil (Gutek et al., 1974; Jones and Lyttleton, 1971). Hence it would be of great benefit if plant breeders could introduce protein precipitants into alfalfa, red clover, and white clover to