Thermal and Base-Catalyzed Hydrolysis Products of the Systemic Fungicide, Benomyl

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The chemical fate of benomyl [methyl 1-(butylcarbamoyl)-2-benzimidazolecarbamate] fungicide in practical use situations was investigated and is described in the present paper. Conversion products were isolated, purified, and subsequently subjected to a number of spectroscopic techniques appropriate for structural characterization. Synthesis routes for obtaining otherwise unavailable reference standards of hydrolysis products are described and a degradative pathway of benomyl to these conversion products is proposed.

Benlate benomyl fungicide (duPont) [methyl 1-(butylcarbamoyl)-2-benzimidazolecarbamate] is registered for use in the control of certain diseases of stone fruits (Environmental Protection Agency, 1971). The wide acceptance of benomyl and its systemic degradation product, designated as MBC (methyl 2-benzimidazolecarbamate) (Kilgore and White, 1970) has justified a broader investigation into the potential commercial uses and consequential fate of these compounds. Research investigations included in this paper were specifically designed to encompass the potential uses of benomyl for the control of fungal diseases associated with the dormant period of stone fruit trees and the postharvest period of the related stone fruits. Investigations into benomyl application during dormancy included *in vitro* and *in vivo* experimentation of a benomyl–Bordeaux mixture. Research into postharvest applications included laboratory-simulated conditions found in heat treatments, commercial spray waxes, and alkaline peeling solutions.

MATERIALS AND EQUIPMENT

Chemicals. Benlate benomyl fungicide (50%) wettable powder and technical benomyl were supplied by E.I. duPont de Nemours & Co., Inc., Wilmington, Del. Methyl-2-benzimidazolecarbamate (SK&F No. 26058) was supplied by Smith Kline & French Laboratories, Philadelphia, Pa. Purity of the chemicals was ascertained by means of thin-layer chromatography and infrared spectroscopy. Reagent-grade chemicals and double-distilled solvents were used in the syntheses and analyses of the benomyl hydrolysis products.

Thin-Layer Chromatograms. Precoated glass plates (silica gel with fluorescent indicator F-254) were purchased from Brinkmann Instruments, Inc., Westbury, N. Y.

Spectrophotometers. Infrared spectra were determined from potassium bromide disks, utilizing a Perkin-Elmer Model 337 spectrophotometer.

Spectrometers. Mass spectra were obtained through the use of a Varian Model M-66 cyclodial double-focusing mass spectrometer. The samples were introduced into the instrument with a direct sample introduction probe. Nuclear magnetic resonance spectra were determined with a Model R-20 Hitachi Perkin-Elmer instrument at 60 MHz with a heated probe. Samples were prepared as deuterodimethyl sulfoxide, with tetramethylsilane (TMS) as an internal standard.

PROCEDURE

Several currently popular disease control measures and routine processing operations involve the use of heat treatments and/or alkaline mixtures, waxes, and solutions. Investigations into the chemical fate of benomyl when used in conjunction with these potential treatments revealed the simultaneous loss of benomyl and the formation of at least three significant hydrolysis products.

Field-Degraded Benomyl. The route of benomyl degradation in the environment, and the isolation, elucidation of structure, and synthesis of MBC (methyl 2-benzimidazolecarbamate) have been reported earlier (Kilgore and White, 1970). The infrared spectrum of MBC is presented in Figure 1B. The nmr spectrum appears in Figure 2B.

Alkali-Soluble Hydrolysis Products. Representative aqueous samples (400 ml) were taken from sprays (8 oz of 50% benomyl to 10:10:100 Bordeaux mixture), waxes, and alkaline peeling solutions containing benomyl or benomyl-treated fruits. Extraneous material was removed from each sample by gravity filtration through Whatman filter paper. The clarified alkaline solutions were acidified to pH 6 with 0.1 *N* hydrochloric acid and the resultant light-grey precipitates were isolated by vacuum filtration. Each filter cake was washed, in turn, with 100 ml of 0.1 *N* hydrochloric acid and 100 ml of distilled water. The acid wash is effective in removing any residual MBC from the filter cakes. The acid washes were combined, neutralized to pH 7 with 1 *N* sodium hydroxide, and extracted with an equal volume (400 ml) of ethyl acetate. The ethyl acetate was filtered through anhydrous sodium sulfate, concentrated in a rotary vacuum evaporator, and analyzed for MBC according to White and Kilgore (1972).

Each filter cake was dried in a vacuum desiccator over anhydrous calcium chloride and then subjected to thin-layer chromatography on silica gel with benzene–methanol (9:1) as the developing solvent. Ethyl acetate was the spotting solvent and a freshly prepared ethyl acetate solution of benomyl served as a reference standard. Compounds on the developed chromatogram were visualized under ultraviolet light (2537 Å). The analytical standard of benomyl demonstrated an R_f of 0.48, while a single spot having an R_f of 0.33 was common to all filter cake extracts. The remaining dried filter cakes were, therefore, combined and prepared for several physical analytical techniques appropriate for structural elucidation.

The infrared spectra of benomyl and its alkali-soluble hydrolysis product are presented in Figure 1, A and C, respectively. A comparison of the conversion product with that of benomyl shows losses of the intense 5.8 μ ester band (-COO-) and the two corresponding asymmetrical and symmetrical stretching bands respective of COC at 7.7 $\sim$ 9.5 μ. Similarly, the conversion product spectrum demonstrates losses of some characteristic CH bending frequencies attributed to the $-COOCH_3$ moiety. These include the symmetrical bending band at 7.3 μ and the asymmetrical bending bands at 6.9, 8.6, 8.8, and 12.6 μ. Upon examination, these facts indicate the loss of the methyl ester group from the parent compound.

Further study of the two spectra shows a loss of the symmetrical stretching NH band at 3.0 μ. This fact, coupled with the appearance of the 2.9 μ band, strongly suggests a cyclic lactam in the free state (Nakanishi, 1962). Supporting evidence includes the loss of the

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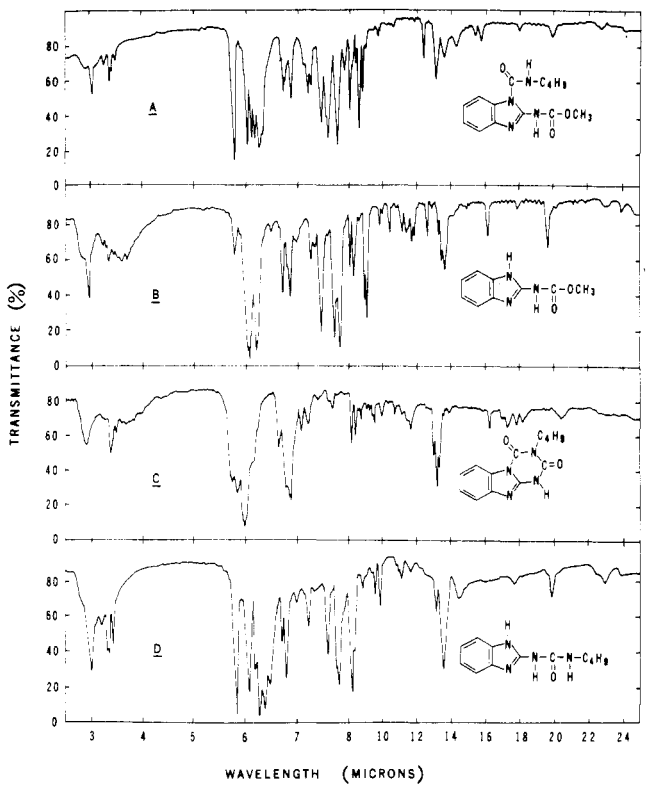


Figure 1. Infrared spectra of (A) benomyl analytical standard, (B) MBC, (C) STB, and (D) BBU.

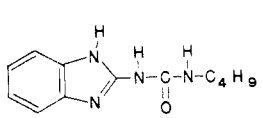
"Amide II and III" bands (6.3 and 7.8 μ) from the secondary amide -CONHR of benomyl, and the stronger or better resolved 2.9 ~ 4.2 μ band due to increased intramolecular hydrogen bonding (H bond with C=O). The "Amide I" band at 6.1 μ is predictably also lost. The strong band at 6.0 μ is probably due to a tertiary amide ($-CONR_2$). Similar characteristics of both spectra include the alkane stretching and bending bands in the 3.4–3.5 μ region, and the ortho-di-substituted aromatic group in the 13.2–13.4 μ region.

The nmr spectrum of the hydrolysis product (Figure 2C) provided supporting evidence to the information derived from infrared analysis. The integral shows a 1-3-2-4-3 relationship excluding the DMSO signal. The absence of a one proton signal for the lactam-lactim tautomer is attributed to exchange. The spectrum indicates chemical shifts relative to TMS at 0.

The mass spectrum of the hydrolysis product was characterized by a molecular ion m/e 32 mass units below the parent compound, benomyl. The even mass number (258.28) and isotopic abundance supports the loss of CH_3OH. The empirical formula derived from the mass abundance tables (Benyon and Williams, 1963) was $C_{13}H_{14}N_4O_2$. Further supporting evidence relative to the empirical formula was obtained from elemental analysis.

Anal. Calcd for $C_{13}H_{14}N_4O_2$: C, 60.40; H, 5.43. Found: C, 60.72; H, 5.38.

The compound was assigned structure I on the basis of physical evidence derived from infrared, nmr, and mass spectra. The calculated mass of the proposed structure 1,2,3,4-tetrahydro-3-butyl-2,4-dioxo-*s*-triazino[*a*]benzimidazole was 258.11. The melting point, or decomposition point, was greater than 300°.



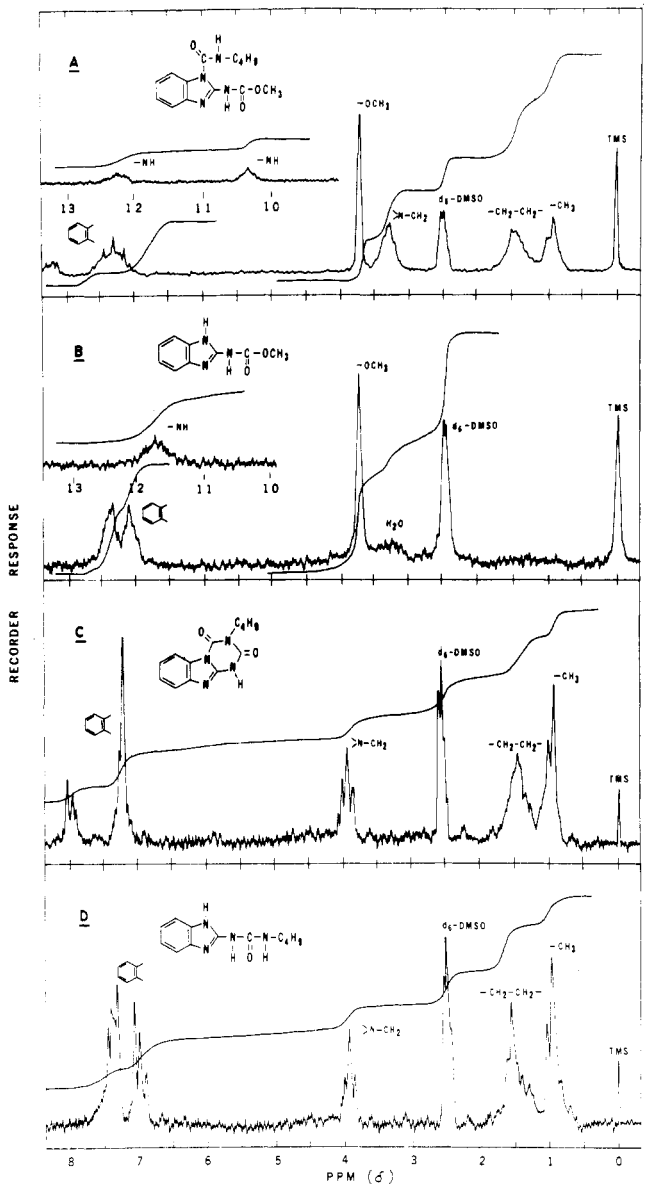


Figure 2. Nmr spectra of (A) benomyl analytical standard, (B) MBC, (C) STB, and (D) BBU.

Synthesis of 1,2,3,4-Tetrahydro-3-butyl-2,4-dioxo-*s*-triazino[*a*]benzimidazole. A scheme for the preparation of the preceding compound, designated as STB, is shown in Figure 3. The synthesis of this compound is analogous to a similar synthesis reported by Pellizarri (1921). Minor changes for adapting Pellizarri's method to the synthesis of STB included the use of *n*-butyl amine rather than aniline in Step II of the synthesis and an additional selective oxidation step utilizing nitrous acid (Step IV). All intermediates formed during the synthesis were isolated and purified before being used in a subsequent step. The final product was recrystallized in the form of fine white needlelike crystals from tetrahydrofuran. The melting point, or decomposition point, was greater than 300°. Final characterization was verified from infrared, mass, and nmr spectra.

Alkali-Insoluble Hydrolysis Product. A second benzimidazole compound was observed to precipitate from standing alkaline solutions of benomyl or STB. Precipitate formation increased proportionately with increasing time, temperature, and/or alkaline strength of the benomyl or STB solution. The precipitate was isolated by vacuum filtration and washed with 100 ml of 0.1 *N* sodium hydroxide and 100 ml of distilled water. The caustic

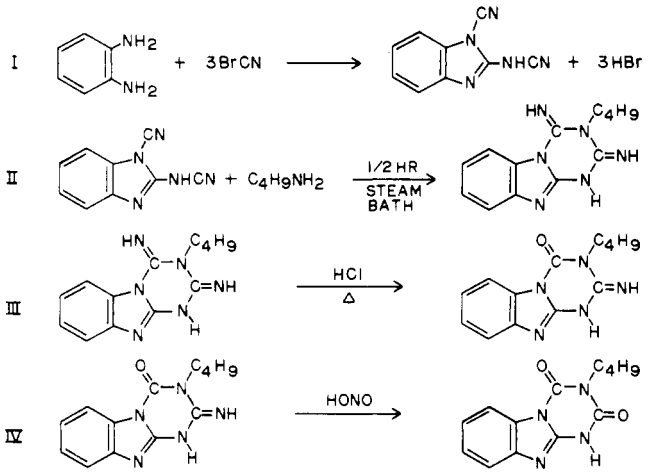


Figure 3. Synthesis of STB.

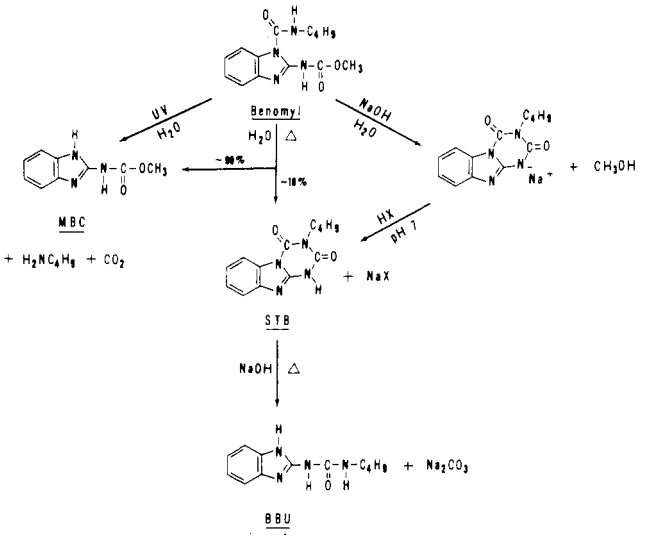


Figure 4. Degradative pathway of benomyl.

wash removed any residual STB or sodium STB from the filter cake. The filter cake was subsequently dissolved in 50 ml of 1.0 *N* HCl and then neutralized to pH 7 with 1.0 *N* sodium hydroxide, and the accompanying precipitate was isolated again by vacuum filtration. The neutralized compound was washed free of sodium chloride with 100 ml of distilled water. The isolated compound was desiccated over anhydrous calcium chloride and then subjected to the same analytical techniques as the previous unknown compound.

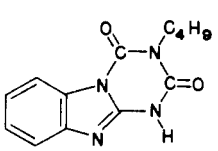
The infrared spectrum of the alkali-insoluble hydrolysis product is represented in Figure 1D. A comparison of this spectrum with the infrared spectrum of benomyl (Figure 1A) shows both a stronger NH band at 3.0 μ and a stronger carbonyl band at 5.8 μ. The presence of these bands together with the apparent "Amide I, II, and III" bands at 6.1, 6.3, and 7.6 μ is indicative of a secondary amide (-CONHR). The alkane stretching and bending bands at 3.4–3.5 μ and the ortho-disubstituted aromatic bands at 13.2–13.4 μ remain unchanged. Finally, the CH bending frequencies (6.9, 8.8, 9.0, and 12.6 μ) attributed to an ester are not present in the infrared spectrum of the hydrolysis product.

The nmr spectrum of the hydrolysis product is reproduced in Figure 2D. The integral shows a 1-3-1-2-4-3 relationship, excluding the DMSO signal. Again, the absence of two nitrogen proton signals might be attributed to exchange. However, other complicating factors included poor dissolution of the compound and the negation of freedom in solvent choice.

The mass spectrum of the hydrolysis product was characterized by the molecular ion, *m/e* 232. The predictable major peaks, *m/e* 159 and 131, due to corresponding losses of the common fragments (-H and $-HNC_4H_9$) and (-H and $-CONHC_4H_9$) were present. The empirical formula was determined from elemental analysis.

Anal. Calcd for $C_{12}H_{16}N_4O$: C, 61.51; H, 6.84; N, 23.95; O, 7.69. Found: C, 61.51; H, 6.90; N, 23.85; O, 7.74.

The compound was assigned structure II on the basis of physical evidence derived from infrared, elemental analysis, nmr, and mass spectrum. The calculated mass of the proposed structure 1-(2-benzimidazolyl)-3-*n*-butyl urea was 232.13. The melting point with accompanying decomposition was 280°.



Preparation of 1-(2-Benzimidazolyl)-3-*n*-butyl Urea. Final proof of structure was accomplished by preparing the urea from STB in a manner similar to a procedure described by Pellizarri (1921, 1924), in which 1,2,3,4-tetrahydro-*s*-triazino[*a*]benzimidazo-2,4-dione was degraded by hot alkali to 1-cyano-2-ureidobenzimidazole. Moist STB (250 mg) from Step IV of the preceding STB synthesis was dissolved in 10 ml of 0.1 *N* sodium hydroxide and clarified by filtration. The filtrate was set aside for 2 weeks to allow for the gradual formation and precipitation of 1-(2-benzimidazolyl)-3-*n*-butyl urea, designated as BBU. Nearly pure BBU was isolated by vacuum filtration, washed with 10 ml of distilled water, and desiccated over anhydrous calcium chloride. The melting point with accompanying decomposition was 282°. Final characterization was determined by spectroscopy.

RESULTS AND DISCUSSION

A Dreiding stereomodel of benomyl illustrates very clearly why the tricyclic compound designated as STB is formed so readily. Bond angles and bond distances are nearly perfect for cyclization. Dilute alkaline solutions catalyze the reaction and yields of monobasic salts of STB are both fast and quantitative. Further degradation to a phenyleneguanyl urea can be minimized by proper handling and storage. The degradative pathway to BBU, MBC, and STB, inclusive, is illustrated in Figure 4. To date little or no information is readily available as to the environmental impact of either STB or BBU.

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