Estimation of Benomyl in the Milligram Range

V. L. Miller,* C. J. Gould, and Elizabeth Csonka

A procedure is described for the estimation of 2.0–12.0 mg of benomyl. The benomyl is extracted from aqueous suspension into chloroform at pH ~10.7 as a copper–benomyl–2-dimethylamino-2-methyl-1-propanol complex. The absorbance of its chloroform solution is measured at 420 nm and compared with the absorbance of known amounts of benomyl. A minor modification will estimate as little as 200 μg of benomyl. The green color of the copper–benomyl–2-dimethylamino-2-methyl-1-propanol complex in chloroform may be compared with permanent standards to estimate the benomyl. This latter approach permits the rapid estimation of benomyl in tanks for treatment of agricultural products without the use of a spectrophotometer.

Aqueous benomyl [methyl 1-(butylcarbamoyl)-2-benzimidazolecarbamate] suspensions of up to 1000 ppm are used as a soak in several agricultural industries to prevent disease caused by *Fusarium, Penicillium,* and other species of fungi (Gould and Miller, 1971; Hilton *et al.*, 1971). For analysis of these suspensions, the usual procedure for several years in this laboratory has been to measure the absorbance of benomyl in dioxane or 2 *N* HCl at 283 nm. Recently, Hilton *et al.* (1971) reported that the measurement of the absorbance of benomyl in ethyl acetate at 281 nm gave satisfactory results. Both of the procedures require the use of an ultraviolet spectrophotometer. A procedure suitable for determination of benomyl as a residue in food products has been reported by Pease and Gardiner (1969). The proposed procedure is suitable to estimate benomyl in the range of 2.0–12.0 mg, either using a visible range spectrophotometer or by comparison with permanent color standards sealed in glass.

MATERIALS AND METHODS

For estimation of benomyl in treatment tanks for bulbs or other agricultural materials, up to 20 ml of a well mixed sample of the tank suspension containing 2.0–12.0 mg of benomyl is placed in a separatory funnel. Water is added if necessary to make a minimum volume of 10 ml. Then 0.2 ml of 2-dimethylamino-2-methyl-1-propanol (DMAMP, from Pfaltz and Bauer) and 10.0 ml of chloroform are added. The separatory funnel is shaken vigorously at once for 1 min. One milliliter of 1% cupric chloride dihydrate is then added to the funnel and the funnel is shaken immediately for another minute. Occasional emulsions may be resolved by allowing the funnel to stand for a short time, by rolling the funnel slowly on its side, or by filtration through phase-separating paper. Severe emulsions requiring centrifugation rarely occur. After the layers separate, the chloroform phase is passed through a small cotton plug in the stem of the funnel to remove suspended aqueous phase. The clear chloroform solution is collected in a 1.00-cm cuvette for spectrophotometric measurement or in an 18-mm o.d. test tube for comparison with permanent standards. The absorbance of the chloroform phase is measured at 420 nm and compared with known amounts of benomyl carried through the same procedure. By comparison of the absorbance at 375 nm rather than 420 nm, the sensitivity of the procedure is increased almost three times. However, for comparison in the 2–12 mg range, either shorter light path cuvettes or the less sensitive measurement at 420 nm is necessary to keep the readings on-scale.

An alternate procedure is to compare the chloroform solution with the permanent standards (Table I). The comparison is facilitated by using a comparator block made from a plastic test tube rack, such as VWR Scientific ±60985-046. Tape 1.3-cm wide is wrapped around the top and bottom of the part of the rack holding the test tubes and the entire rack is then sprayed with black paint. Comparison is made against a white background under a good light.

The range of the method may be extended from 200 μg to 2.00 mg of benomyl. The procedure is exactly the same, except that 5.0 ml of chloroform is used with 0.1 ml of DMAMP and 0.5 ml of the copper solution. The absorbance is determined at 375 nm and compared with the absorbance of standard benomyl.

To determine the usefulness of the proposed procedure, suspensions of commercial benomyl (from Benlate Benomyl Fungicide, 50% WP) were prepared at approximate concentrations used in the flower bulb industry. The concentration of benomyl was determined by spectroscopy at 420 nm, by visual observation using the artificial color standards, and spectroscopy at 283 nm in 2 *N* HCl. Materials added to bulb-treating tanks by the bulb growers, such as formalin or aldrin wettable powder, and materials inadvertently present such as soil, bulb husks, and crushed bulbs were added and the analyses repeated 2 days later. Typical results before and after addition of the indicated contaminants to 200 ml of benomyl suspension are shown in Table II.

RESULTS AND DISCUSSION

The artificial standards (Table I) were developed with inorganic ions to present a permanent color system that would not fade with time. Excess nitric acid and chromic acid cleaned tubes were used to avoid hydrolysis or reduction of the dichromate in the sealed tubes. The colored chloroform solution of the Cu–benomyl–DMAMP complex was stable for several hours, but could change on standing overnight. Rarely would a cloudiness develop on standing even a few minutes. This cloudiness was due to extraneous materials in treating tank samples or in experimental samples containing methyl 2-benzimidazolecarbamate (MBC), a fungicidally active decomposition product of benomyl (Jhooty and Singh, 1972).

If cloudiness occurred, pressure filtration through several layers of glass fiber filter paper was used to effect clarification. In practice, comparison was made at once with the permanent standards.

The data in Table II indicate that the results from the visual comparison with the permanent standards or by determination of the absorbance at 420 nm will give re-

Departments of Agricultural Chemistry and Plant Pathology, Washington State University, Western Washington Research and Extension Center, Puyallup, Washington 98371.

Table I. Composition of Permanent Standards for Estimating Benomyl Concentrations. The Indicated Volumes of the Solutions for Each Standard Are Combined in a 18-mm o.d. Test Tube. The Tube is Closed with a Melted Glass Seal

Reagent	Benomyl equivalent of standard, mg					
	12.0	10.0	8.0	6.0	4.0	2.0
H_2O	4.90 ml	5.00 ml	5.60 ml	6.50 ml	7.10 ml	8.25 ml
37.5% w/v[a] $Ni(NO_3)_2 \cdot 6H_2O$	3.78	3.20	2.49	1.92	1.22	0.64
6.12% w/v[a] $Co(NO_3)_2 \cdot 6H_2O$	1.20	1.30	1.30	1.20	0.98	0.45
0.01% w/v $K_2Cr_2O_7$	0.35	0.47	0.56	0.65	0.68	0.66

[a] Contained 2.0 ml of 16 *N* nitric acid/100 ml.

Table II. Ben omyl Concentration in Suspensions before and after Addition of Contaminants

Before addition of contaminants			After addition of contaminants			
Ppm from			Ppm from			
Absorbance at		Visual	Absorbance at		Visual	Contaminant
283 nm	420 nm	comparison	283 nm	420 nm	comparison	added/200 ml of suspension
1115	1070	1100	1111	1110	1100	Aldrin, 0.9 g
868	887	950	907	895	950	Formalin, 1.0 ml
877	828	900	870	820	900	Soil, 3.0 g
914	890	900	931	870	950	Soil, 1.0 g + Husk, 1.0 g
859	867	900	870	838	900	Bulb, 5.0 g + Soil, 1.0 g
590	588	650	636	570	600	Peat soil, 1.0 g
1234	1185	1200	1136	1135	1150	Formalin, 1.0 ml
632	695	650	685	640	650	Soil, 3.0 g
312	344	350	359	320	350	Husk, 2.0 g
865	910	900	882	848	900	Husk, 1.0 g + Formalin, 1.0 ml

Table III. Amines Grouped by Absorbance at 375 nm from the Reaction with 5 mg of Benomyl

Group I, absorbance >1.000	Group II, absorbance 0.500–1.000	Group III, absorbance 0.200–0.500	Group IV, absorbance <0.200
1-Dimethylamino-2-propanol	Piperidine	Tri-*N*-octylamine	2(Methylamino)ethanol
3-Amino-1-propanol	3-Dimethylamino-1-propanol	Dimethylamino-cyclohexane	2-Amino-2-methyl-propanol
2-Dimethylaminoethanol		Triethylamine	Triethylenetetramine
2-Dimethylamino-2-methyl-1-propanol (DMAMP)		Ethylamine	1-Ethanolamine
		Diethylamine	1-Amino-2-propanol
		Tetramethylguanidine	Dimethylaniline
		Methyldiethanolamine	Tetramethylenepentamine
		Methylethanolamine	2-Aminobutane
		Morpholine	Diethanolamine
		Piperazine	

sults satisfactory for use in analysis of treating tank suspensions as used in the bulb industry. The common extraneous materials used in bulb treatment or inadvertently present in the treating tanks did not interfere appreciably.

The reaction of benomyl, copper ion, and DMAMP as described occurred at pH 10.5–10.7. Some color was produced and extracted at a lower pH, but for maximum color the pH should be $\geq$ 10.5. The estimation could be made by adding the copper chloride solution before the first extraction. However, the color produced was less and erratic. Methylene chloride or methylchloroform was usable for the extraction instead of chloroform. Although methylene chloride extracted slightly more color than chloroform, this advantage did not outweigh the other advantages of chloroform. Amyl alcohol was not satisfactory as an extraction solvent. DMAMP, present in a considerable excess, not only was a component of the extracted complex but also acted to adjust the pH. The ratio of Cu to DMAMP was important. Thus, use of either 0.2 ml of DMAMP and 1.00 ml of copper chloride solution or 0.3 ml of DMAMP and 1.50 ml of copper chloride solution gave the same color intensity with the same amount of benomyl. However, an increase in the DMAMP–Cu ratio decreased the amount of colored compound extracted. Large amounts of copper ion increased the absorbance of the chloroform solution erratically. One milliliter of 1% cupric chloride dihydrate used in the described procedure contained only slightly more than the minimum amount of copper ion necessary for 12 mg of benomyl. The addition of 1.0 g of sodium chloride prior to the first extraction increased the absorbance ~10%, but this was not considered enough to warrant the addition of this extra reagent.

The structure of the extracted complex was not determined. The copper–benomyl molar ratio was 1:1. The ratio was determined by extraction of the copper from a chloroform solution of the compound containing a known amount of benomyl using *N* HCl, and the copper was determined by the procedure of Williams and Morgan (1954). The amount of DMAMP in the extracted compound was not determined.

Other amines substituted for DMAMP in the described procedure produced variable amounts of color (Table III). The amines producing the most color were all two or three carbon primary or secondary alcohols with a β-amino or an α-dimethylamino group. The other amines gave less color, usually yellow, and in general the amines were in groups similar to but not exactly the same as those found by Miller *et al.* (1973) to react with thiabendazole [2-(4′-thiazolyl)benzimidazole]. DMAMP was selected for use in the procedure because the color intensity was greater with

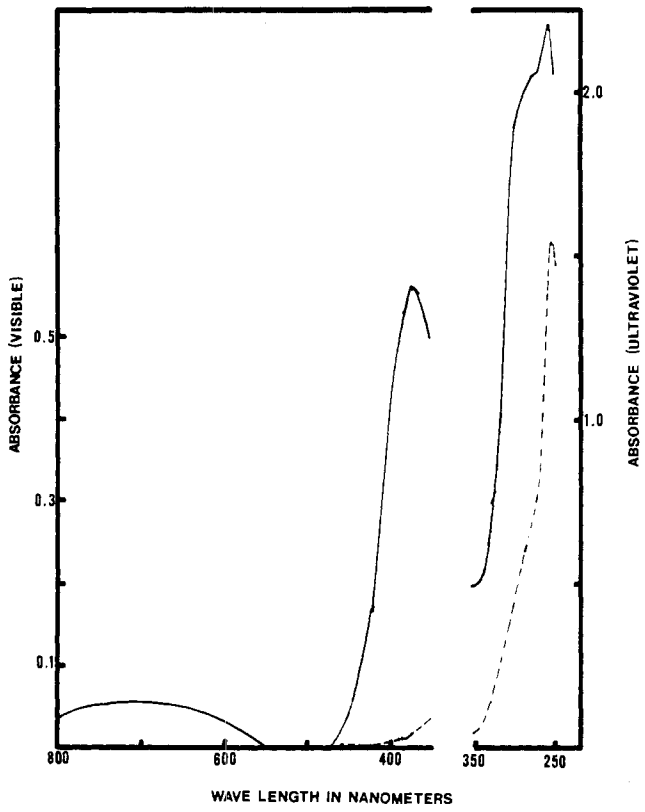

Figure 1. Absorption spectra of the copper–benomyl–2-dimethylamino-2-methyl-1-propanol complex (——) and copper–2-dimethylamino-2-methyl-1-propanol complex (----) in chloroform.

this amine than any of the others tested. Also, an absorption peak at 375 nm was observed. Further, some of the amines tested gave chloroform solutions that were difficult to clarify due to colloidal material.

Thiabendazole carried through the procedure produced over twice as much absorbance as did the same weight of benomyl. MBC produced approximately the same absorbance as did an equal weight of benomyl up to ~2 mg. With larger amounts, the amount of absorbance produced decreased, suggesting incomplete reaction. The solubility of MBC in chloroform was 130 μg/ml, compared to 7.1 mg of benomyl/ml. The solubility difference may account for the lack of color produced with the larger amounts of MBC. Also, the solubility of the copper–MBC–DMAMP complex was probably low in chloroform, since by substituting 1-dimethylamino-2-propanol for DMAMP, the reliable range of the reaction with MBC could be doubled.

Peterson and Edgington (1969) reported that benomyl in water decomposed completely to MBC in a few days. White *et al.* (1973) reported that other materials than MBC are formed in the degradation of benomyl. However, the difference in the color reaction by MBC and benomyl would suggest that the hydrolytic reaction was less rapid than reported or that other breakdown products were involved in the color production. After benomyl stood 2 days in aqueous suspension at room temperature, the analyses by the proposed procedure remained essentially unchanged (Table II). Neither benzimidazole or 2-aminobenzimidazole reacted to produce an extractable colored compound alone, nor did they increase the absorbance when mixed with benomyl.

Extraneous color can interfere with this method. Therefore, it is recommended that potential users run a blank with benomyl formulation and DMAMP, but without copper chloride solution. The blank should be essentially colorless if comparison is made with the permanent color standards. Much yellow color in the extract will make the comparison with the permanent color standards difficult or impossible. If a spectrophotometer is used, a correction for the blank may be made.

Standard benomyl may be dissolved in chloroform but should be prepared fresh every few days since insoluble material soon appeared (Kilgore and White, 1970). Benomyl was soluble in dimethylsulfoxide, dimethylformamide, and methanol. However, much lower absorbance was observed when these solvents were used in the preparation of standard benomyl.

As noted in a similar procedure for thiabendazole (Miller *et al.*, 1973), the green color observed with benomyl is believed to be a blend of the blue and yellow colors of the spectrum (Figure 1). The Cu–benomyl–DMAMP complex absorbed appreciably in the 800–600 nm range, and has no absorption between 540 and 480 nm. A peak was observed at 37.5 nm, and again in the ultraviolet below 300 nm. However, the background absorption from the reagents was appreciable in the ultraviolet.

The accuracy of an individual test using the permanent standards cannot be expected to be better than ±50 ppm. In Table II, the means of the three methods of estimation are within 3% before addition of the extraneous materials and within 5% after.

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