

SYNTHESIS OF DEUTERIUM LABELLED BENOMYL AND CARBENDAZIM RESIDUE STANDARDS

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SUMMARY

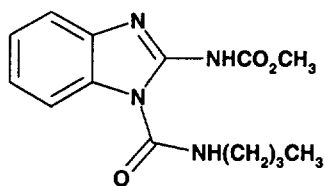
Three environmental degradation products of benomyl possessing tetradeutero-ring substitutions were synthesized for use in quantitative analysis of trace residues. The common precursor, 1,2-phenylenediamine, was exhaustively deuterated upon heating in acidic aqueous media. Further synthetic transformations to 2-aminobenzimidazole-4,5,6,7- $^2\text{H}_4$, carbendazim-4,5,6,7- $^2\text{H}_4$, and 3-butyl-1,3,5-triazino-[1.2a]benzimidazole-2,4(1H,3H)-dione-6,7,8,9- $^2\text{H}_4$ proceeded with complete retention of the ring substituted deuterium.

Key Words: benomyl, carbendazim, 1,2-phenylenediamine, deuterium labeling

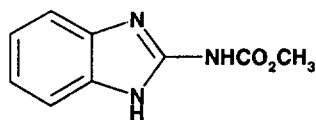
INTRODUCTION

Benomyl and carbendazim are active ingredients in commercially important agricultural fungicides useful for the prevention and treatment of diseases on a variety of crops and ornamental plants. An enhanced method for crop residue analysis by LC/MS was sought that would compensate for matrix effects encountered with atmospheric pressure interfaced electrospray ionization detection technique currently employed.^{1,2} Isotopic labeling is an accepted practice for accurate quantitation of analytes in complex matrices;^{2,3} however, the use of isotopically labelled standards may be limited by the expense and technical challenges of synthesis of multiple analytes for a particular parent substance. The following discussion focuses on the

efficient synthesis of tetra-deutero analogs of three typically observed residues from field applied benomyl-containing fungicides. One of these residual substances is methyl 2-aminobenzimidazole carbamate (MBC, carbendazim).



Benomyl

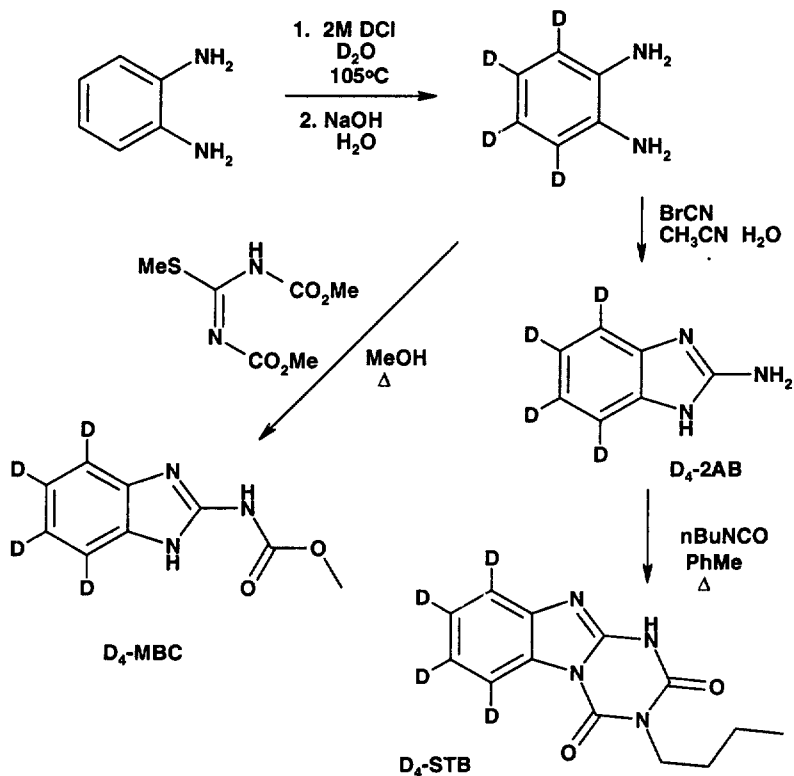


Carbendazim

RESULTS AND DISCUSSION

The exchange of ring hydrogens in the ortho and para positions of anilines is known to occur in acidic media.^{4,5} Likewise, in basic media, the analogous hydrogens in phenol can be exchanged for deuterium.^{5,6} Through a similar mechanism, it was postulated that 2-aminobenzimidazole (2AB) or methyl 2-aminobenzimidazole carbamate (MBC) would be capable of analogous reactions to afford the fully ring substituted deuterium analogs. However, attempts to exchange the ring hydrogens with deuterium in either 2AB or MBC using either strongly acidic or basic media, both aqueous and non-aqueous (tBuOK/tBuOD), failed to afford any observable hydrogen replacement. This would suggest that the positive or negative charge that can be generated on the guanidino moiety in these compounds is not appreciably delocalized into the benzene ring, as it is in anilinium and phenolate ions.

Analogous to its 1,4-substituted isomer⁷, though under modified conditions, 1,2-phenylenediamine was subjected to heating (105°C) in acidic aqueous media (2N DCl/D₂O) and found to readily exchange all eight hydrogens. Recycling of the initial product one time afforded >98% atom ²H₄ substitution, after the amino deuterons had been exchanged back to protons during aqueous work-up at ambient temperature. Conversion of the [²H₄]-1,2-phenylenediamine to deuterated 2AB, MBC and STB was accomplished via established routes for the unlabelled analogs as shown.



Mass spectroscopy (ESI⁺) on the three labelled products indicated that subsequent reactions of [²H₄]-1,2-phenylenediamine proceeded without loss of the ring-substituted deuterium atoms. Generally only 1-2% of the ²H₃ labelled compounds were observed, unchanged from starting material, with no detectable masses corresponding to 0, 1, and 2 deuterium substitutions. Thus, these compounds can be used as quantitative reference standards for the corresponding benomyl residues without interference from their naturally occurring ¹³C isotopomers. Further validation for their use as stable reference materials was indicated by the inability of MBC and 2AB to undergo nuclear exchange reactions under extreme conditions, as discussed above. A labelled reference standard of benomyl itself was not required since benomyl is not typically observed as a crop residue due to its rapid breakdown in the environment. The two reference standards of deuterated carbendazim (MBC)

and 2AB would presumably be of utility for the analysis of residues of carbendazim containing fungicides as well. A more complete discussion of this specific analytical technique will be published elsewhere.

EXPERIMENTAL

Chemicals. 1,2-Phenylenediamine, cyanogen bromide/acetonitrile solution and other reagents were purchased from Aldrich Chemical Co. Deuterium oxide (99.9% D) and deuterium chloride (35% in D₂O, min 99% D) were purchased from Isotec, Inc.

Spectroscopy. Mass Spectra were obtained on a Finnigan LCQ ion trap spectrometer in electrospray positive ion mode. NMR spectra were obtained on a Varian INOVA 500 MHz spectrometer.

1,2-Phenylenediamine-3,4,5,6-²H₄. A mixture of 1,2-phenylenediamine (15 g, 0.14 mol), deuterium oxide (200 g) and 35% deuterium chloride (50 g, 0.47 mol) was heated to reflux (ca 105°C) under nitrogen atmosphere for 18 hr. The solution was evaporated to dryness, then re-dissolved in more D₂O (155 g) plus DCl (40 g of 35%) and reheated to reflux for another 18 hr. After evaporation to dryness under reduced pressure, the greenish solid residue was dissolved in water (250 mL), treated with activated charcoal for 10 min, and filtered through Celite® with water rinse. The filtrate was chilled in ice and basified with aq. NaOH (50%), then extracted with dichloromethane (4 x 100 mL), dried (Na₂SO₄) and evaporated to a beige crystalline solid, 13.1 g (84%) of 1,2-phenylenediamine-3,4,5,6-²H₄; mp 102-104°C. Mass Spectrum (ESI+): 113 (MH⁺, 100), 112 (<2) *m/z*. The isotopic ratio of 98% *d*₄ to <2% *d*₃ remained constant after standing for several days in aqueous solution.

2-Aminobenzimidazole-4,5,6,7-²H₄ (D₄-2AB). A solution of 1,2-phenylenediamine-3,4,5,6-²H₄ (4.75 g, 0.042 mol) in 20 mL acetonitrile plus 20 mL water was chilled to 0°C and treated with cyanogen bromide solution (8.7 mL of 5M in CH₃CN, 0.043 mol) via dropwise addition over 10 min, keeping T < 12°C. After stirring for 2 hr in the ice bath, the solution was evaporated to 1/3 volume, diluted with water (50 mL),

treated with activated charcoal, and filtered through Celite®. The filtrate was chilled and neutralized with one equivalent of 2N NaOH (21.5 mL) whereupon a solid crystallized out, and was collected by filtration. Solids were rinsed with minimal water then ether and dried under vacuum to 4.4 g of beige plates, mp 227-229°C. Mass Spectrum (ESI+): 138 (MH⁺, 100), 137 (<2) *m/z*, indicating >98% atom D₄. ¹H-NMR (DMSO-*d*₆) 6.1 (br s, NH₂), 10.7 (br s NH) ppm; ²H-NMR (DMSO-*d*₆) 6.85 (br s), 7.20 (br s) ppm; ¹³C-NMR (DMSO-*d*₆ 50°C) 156.5, 140 (br), 119.8 (br), 112 (br) ppm

Methyl 2-Aminobenzimidazolecarbamate-4,5,6,7-²H₄ (²H₄-Carbendazim) (D₄-MBC). A mixture of 1,2-phenylenediamine-3,4,5,6-²H₄ (6.0 g, 0.054 mol) and 1,3-bis(methoxycarbonyl)-2-methyl-2-thiopseudourea (11.3 g, 0.055 mol) in 75 mL methanol was heated to reflux under nitrogen for 18 hr. The mixture was cooled to ambient temperature and the solid product collected by filtration, and rinsed well with methanol to afford 9.33 g (90%) of white crystalline powder, mp > 275°C. Mass Spectrum (ESI+) 196 (MH⁺, 100), 195 (<2) *m/z*, indicating >98% atom D₄. ¹H-NMR (DMSO-*d*₆) 3.78 (s, 3), 11.65 (br s, NH x 2) ppm; ²H-NMR (DMSO-*d*₆) 7.05 (br s), 7.40 (br s) ppm; ¹³C-NMR (DMSO-*d*₆) 155.3, 147.7, 135.8, 120.4, 52.3 ppm.

3-Butyl-1,3,5-triazino[1,2a]-benzimidazole-2,4(1H,3H)-dione-6,7,8,9-²H₄ [D₄-STB]. A suspension of 2-aminobenzimidazole-4,5,6,7-²H₄ (5.0 g, 0.37 mol) in 120 mL toluene was treated with a large excess of n-butylisocyanate (21.4 mL, 0.19 mol) and heated to reflux for 10 hr. Evaporation to a dark viscous oil followed by trituration with n-chlorobutane to afford 2.9 g of a white powder after drying. A second crop isolated from the filtrate proved to be a butylisocyanate adduct of the product as determined by LCMS. To convert this back to desired product, the combined solids and filtrate were condensed and hydrolyzed by mixing with equal amounts of methanol, acetonitrile and water with ca 0.50 mL conc HCl and heated on the steam bath for several hours until the adduct was consumed as monitored by TLC (EtOAc/hexanes 4:1). The solids that formed were collected by filtration and extensively rinsed with methanol water 3:1, acetone and ethyl acetate. A second

trituration of these solids with hot acetone/ethyl acetate 1:1 afforded 5.5 g of additional product. This crop and the original crop were combined and triturated with hot EtOAc/methanol 20:1 to afford a total of 8.0 g (83%) of white crystalline powder, mp > 275°C. Mass Spectrum (ESI+) 263 (MH⁺, 100), 262 (<2) *m/z*, indicating >98% atom D₄. ¹H-NMR (DMSO-*d*₆) 0.93 (t, J=7 Hz, 3), 1.35 (m, 2), 1.6 (m, 2), 3.85 (q, J=7 Hz, 2), 12.75 (NH) ppm; ²H-NMR (DMSO-*d*₆) 7.4 (br), 8.0 (br) ppm; ¹³C-NMR (DMSO-*d*₆) 13.7, 19.5, 29.4, 40.9, 113.1, 114.0, 121.9, 124.7, 135.9, 146.7, 148.4, 151.7 ppm.

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