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Note

Fungitoxic alkyl 2-benzimidazolecarbamates and some thin-layer chromatographic detection reagents

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The fungitoxic principles of the systemic fungicides benomyl, thiophanate-methyl and thiophanate have been found to be methyl 2-benzimidazolecarbamate (MBC) or ethyl 2-benzimidazole carbamate (EBC)[1,2]. Thin-layer chromatographic (TLC) methods for separating MBC from other fungicidal compounds have been described by Von Stryk[3] and Baker *et al.*[4]. An interesting discrepancy between their observations is that whereas Von Stryk described the occurrence of an intense blue colour when spots of MBC were sprayed with 2,6-dichloro-*p*-benzoquinone-4-chlorimine (N-2,6-trichloro-*p*-benzoquinonimine), this result could not be reproduced by Baker *et al.*[4] using TLC sheets obtained from a different manufacturer. Some experiments carried out on EBC, MBC and their common hydrolysis product, 2-aminobenzimidazole (2AB), which could possibly explain the discrepancy, are presented in this paper.

EXPERIMENTAL

A paper-lined chromatographic development tank, a 10-μl micropipette, a chromatographic sprayer and a tank for exposing the TLC sheets to gaseous reagents were used. The TLC sheets were Eastman Chromatogram 6060 sheets (Eastman-Kodak, Rochester, N.Y., U.S.A.) coated with silica gel containing a fluorescent indicator, and Merck TLC aluminium sheets 5554/0025 coated with silica gel F_{254} (E. Merck, Darmstadt, G. F. R.).

The analytical standards used were EBC and MBC supplied by Nippon Soda Co., Tokyo, Japan, and 2AB obtained from DuPont, Wilmington, Del., U.S.A.

Procedure

Spots containing 0.1, 0.3, 1.0 and 3.0 μg of EBC, MBC and 2AB were placed on the sheets and the chromatograms were developed using a 10% solution of methanol in benzene. After drying and observation of UV fluorescence, the following techniques were tried: spraying with a 0.5% solution of 2,6-dichloro-*p*-benzoquinone-4-chlorimine in cyclohexane and in acetic acid, a 1% solution of 2,6-dibromo-*p*-benzoquinone-4-chlorimine in the same solvents and aqueous sodium hypochlorite solution (0.3% free chlorine), and exposure to chlorine and bromine vapour.

RESULTS

The spots of EBC and MBC reacted in exactly the same way to the chromogens. Spraying with the cyclohexane solution of the halogenated benzoquinoneimines made the spots containing 1 and 3 μg of carbamate appear on the Eastman sheets as weak blue areas. Heating at 100° increased the intensity of the colour, so that even the spots of 0.3 μg of carbamate became visible for a moment, but then the blue colour faded, and after 10 min it was difficult to see spots of 1 μg of carbamate. On the Merck sheets, the same amounts of the carbamates did not respond to these chromogens.

The carbamate spots could be made visible by exposing the sheets to bromine vapour, whereupon renewed exposure to UV light resulted in a greyish brown colour. The method is not sensitive, and 1 μg could scarcely be seen.

When 2AB was sprayed with a cyclohexane solution of the benzoquinoneimines, blue spots developed similar to those obtained by spraying the carbamates, but the colour was much more intense and also appeared on the Merck sheets. Heating at 100° had essentially the same effect on the spots of this compound as on those of the carbamates. With the stronger colour, it could be seen that during fading the colour changed gradually from blue to brownish yellow. At the moment of maximum intensity, spots containing 0.1 μg could easily be seen. If the chromatograms were taken out of the oven when the colour was at its most intense, the change proceeded more slowly, but it was speeded up considerably by exposure to UV radiation. Spots of 2AB could also be made visible by spraying with sodium hypochlorite solution. The colour did not differ from that obtained by spraying with the benzoquinoneimines. Exposure to chlorine or very dilute bromine vapour gave spots with the same colour. Increasing the concentration of bromine vapour resulted in a yellowish brown colour. The colour intensity of the spots developed by the halogens or hypochlorite solution could not be increased by heating.

Spraying with benzoquinoneimines in acetic acid did not lead to any visible spot for any of the benzimidazole derivatives tested.

DISCUSSION

The chromogenic spray reagent 2,6-dichloro-*p*-benzoquinone-4-chlorimine, and the corresponding ring-substituted bromine compound, gave a spot with 2AB similar to the blue colour produced by spraying with aqueous sodium hypochlorite solution, or by exposing 2AB to chlorine or dilute bromine vapour. Hence it can be suggested that the true chromogenic part of the benzoquinoneimine molecule is the highly reactive chlorine atom bound to the nitrogen atom.

More than 50 years ago, Pellizzari and Gaiter[5] observed that the addition of hypochlorite or hypobromite to 2AB in aqueous solution caused the appearance of an intense blue colour that changed to yellowish brown. This phenomenon has been utilized in a quantitative colorimetric method in this laboratory[6].

From the point of view of quantitative TLC analysis, the development of colour by exposure to gaseous reagents is preferable to spraying, as a gas can be more evenly distributed. Vogel *et al.*[7] used the yellowish brown colour obtained by bromination on TLC plates to determine 2AB as a hydrolysis product of benomyl. This is probably

the most practical method for the semiquantitative determination of this important fungicide using direct observation of the thin-layer chromatograms. The blue colour obtained by exposing 2AB to chlorine gas is likely to give an even more sensitive method.

Both the result of Baker *et al.*,[4] and to a certain extent also that of Von Stryk[3], concerning the colour development of MBC with benzoquinone chromogen, have been reproduced here. This indicates that the discrepancy discussed earlier is due to the choice of the TLC sheets. As the colour obtained on Eastman sheets is similar to that which is developed with 2AB, the most probable mechanism is a hydrolysis of the carbamates to 2AB under the influence of the material of this particular sheet. Hydrolysis to 2AB occurs in plants[8] and soil[9], indicating that extreme alkalinity is not necessary. It still remains to be explained how Von Stryk succeeded in converting so much of the MBC on his TLC sheet into 2AB so that he could obtain the low detection limit he reported.

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