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The Metabolism of the Systemic Fungicide Carboxin (Vitavax) by *Rhizopus japonicus*

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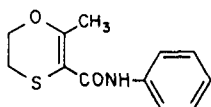
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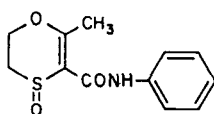
Cultures of *Rhizopus japonicus* in synthetic glucose medium convert Carboxin into Carboxin sulfoxide and Carboxin sulfone. In steep medium, under partially anaerobic conditions, Carboxin sulfoxide and another metabolite, which is formed by the addition of the elements of water, are the main products.

INTRODUCTION

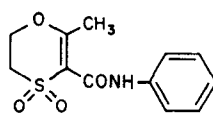
1,4-Oxathiin derivatives constitute a large class of compounds with systemic fungicidal activity.^{1,2} Carboxin (I; Vitavax; 2,3-dihydro-5-carboxanilido-6-methyl-1,4-oxathiin) and Oxycarboxin (III; Plantvax; 2,3-dihydro-5-carboxanilido-6-methyl-1,4-oxathiin-4,4-dioxide) were introduced in 1966³ and, particularly the former, have since been used extensively in seed treatment for the control of a number of fungal plant diseases.



I



II



III

Studies on the breakdown of Carboxin in soil and water and its metabolism in plants and animals⁴⁻⁸ showed relatively rapid conversion to the sulfoxide (II) in all systems and the formation of small quantities of the sulfone (III, Oxycarboxin). No further intermediates in the breakdown and metabolic excretion were found, with the exception of some uncharacterized anilide complexes, and of several unidentified compounds which were found on thin-layer chromatograms of extracts from Carboxin-treated cotton seedlings.⁹ Since phycomycetes from the genera *Rhizopus*^{10,11} and *Mucor*¹² were found to metabolize aromatic fungicides the metabolism of Carboxin by pure cultures of *Rhizopus japonicus* was investigated in this study.

MATERIAL AND METHODS

Chemicals and Instrumentation

Carboxin, Carboxin sulfoxide, Carboxin sulfone and labeled Carboxin (1-phenyl-¹⁴C; specific activity 2.35 μ Ci/mM) were obtained from Uniroyal Research Laboratories, Guelph, Canada, and from Uniroyal Chemical, Naugatuck, U.S.A.

The TLC scanner for measurement of radioactivity was model LB 2723, Frieske, Karlsruhe, Germany. All other instruments used, and conditions for GC and TLC were those described earlier.^{10,11}

Culture Methods and Growth Conditions

Rhizopus japonicus was grown (i) in a synthetic glucose (Wegener) medium¹⁰ on a shaker providing aerobic conditions (temperature, 30°; pH range during growth, 6.0–7.5); and (ii) in a steep medium¹³ without aeration through shaking, providing partially anaerobic conditions (temperature, 30°; pH range during growth, 5.5–2.8). Sterile controls were run for both systems, the pH in the steep medium, however, was not adjusted to acid values.

Analytical Procedures

Ten-milliliter portions of the culture medium were extracted with chloroform twice a week and estimated for fungicide degradation and formation of metabolites by u.v. analysis (for methodology see ref. 10).

Isolation of Fungicide Metabolites

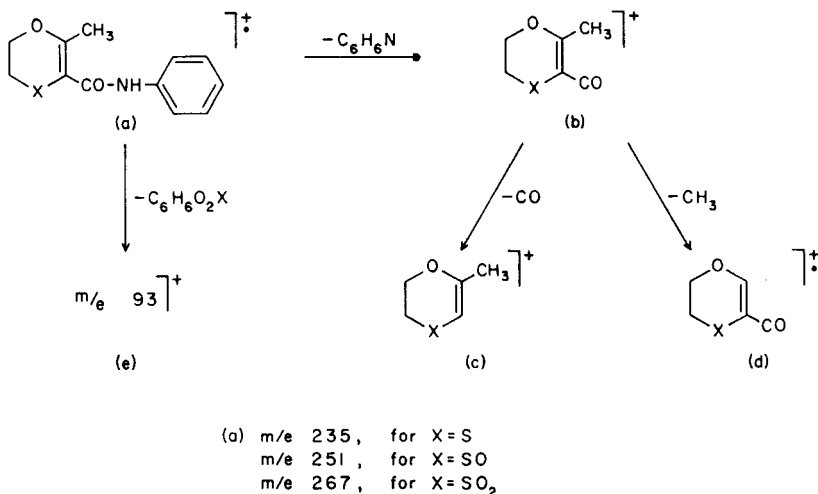
After removing the mycelial mass by filtration the medium (500 ml) was extracted with chloroform (1000 ml). The chloroform extract was dried (Na_2SO_4), the volume reduced to about 5 ml by rotary evaporation and

streaked on to a preparative silica TLC plate (0.5 mm thickness). After elution with chloroform-benzene (9 : 1) the bands corresponding to the metabolites were removed from the plate and the silica was extracted with chloroform. Both metabolites were recrystallized from chloroform-carbon tetrachloride (1 : 1) after the solvent had been evaporated. Extraction and purification of the unidentified metabolite (M-3) was carried out in an identical manner except that the material could not be obtained in crystalline form.

RESULTS AND DISCUSSION

Identification of Metabolites, Physical Data

Carboxin sulfoxide and Oxycarboxin were identified as metabolites by comparison with authentic material (m.p., co-chromatography on TLC, n.m.r. and mass spectra). The mass spectrum of M-3 indicated a mixture of compounds with molecular ions appearing at m/e 269, 285 and 253. The latter ion was due to the major component of the mixture and indicated the addition of the elements of water to Carboxin. Due to insufficient material and to the apparent co-chromatography of the impurities under the TLC and GLC conditions used no further structure elucidation was carried out.



The mass spectrum of Carboxin gives a fragmentation pattern (scheme 1) typical of anilide derivatives. Cleavage of the molecular ion at the amide bond gives an intense fragment ion at m/e 143 for the heterocyclic moiety (b) expels both CO and $\text{CH}_3 \cdot$ to give ions (c) (m/e 115) and (d) (m/e 128), respectively. A less intense but characteristic peak occurs at m/e 93 for the ion at m/e 251 and the resulting fragment ions (b), (c), and (d) appeared at

16 mass units higher than in Carboxin, whereas the anilino fragment at m/e 93 was unchanged. Similarly, for Carboxin sulfoxide ($M^+ = m/e$ 267) the fragment ions (b), (c), and (d) occurred at 32 mass units higher than in Carboxin and these ions also show further expulsion of oxygen, a reaction typical of sulfones. The spectra of the oxygenated metabolites were identical to authentic samples of the sulfoxide (II) and the sulfone (III).

TABLE I
N.m.r. spectra of Carboxin and metabolites

Compound	Group (τ value)			
	CH ₃ -	-CH ₂ -S	-CH ₂ -O	Aromatic
Carboxin	7.72	7.0	5.60	2.3-3.0
Carboxin sulfoxide	7.65	6.58	5.47	2.3-3.0
Oxycarboxin	7.70	6.58	5.25	2.3-3.0

TABLE II
Physical data for Carboxin and metabolites

Compound	TLC† R_f values		U.v. λ_{\max} in CHCl ₃ (nm)	m.p.	GLC‡ Retention time (min)
	A	B			
Carboxin (I)	0.67	0.90	292	97-98	3.6
Carboxin-sulfoxide (II)	0.08	0.09	250	121-122	—
Oxycarboxin (III)	0.22	0.50	266	127	7.7§
M-3 (IV)	0.28	0.60	243	—	3.9

†A, CHCl₃-benzene = 9 : 1

B, CHCl₃-ethylacetate = 1 : 1.

‡GLC—conditions: column, 220°; detector, 260°; injector 250°. All other conditions as previously described (refs. 10, 11).

§Column temperature 240°.

The n.m.r. spectral data (Table I) also confirm the above results, since the chemical shift values for Carboxin, Oxycarboxin and Carboxin sulfoxide are all significantly distinctive. Some other physical data are given in Table II.

Metabolism of Carboxin under Aerobic Conditions

With the beginning of the exponential growth of *Rhizopus japonicus* Carboxin sulfoxide (II; the major metabolite) and Oxycarboxin (III) could be detected

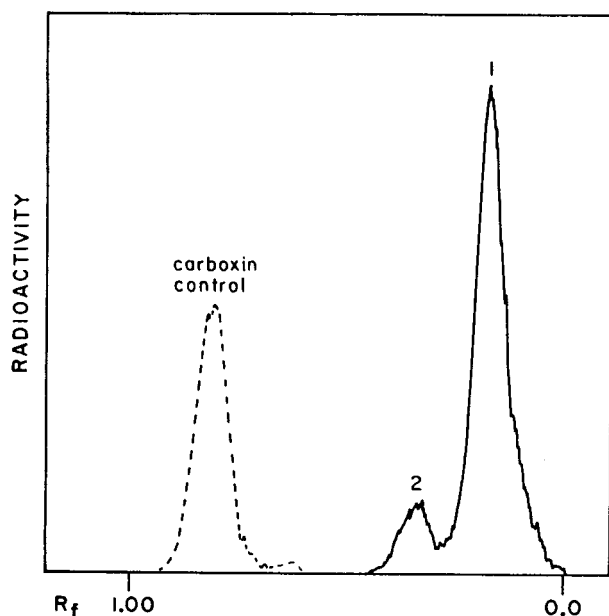


FIGURE 1 Radio-TLC scan of the chloroform extract of culture medium (Wegener) in which *Rhizopus japonicus* had been growing for four days in the presence of ^{14}C -labeled Carboxin. 1: Carboxin-sulfoxide. 2: Oxycarboxin. Carboxin control: unchanged Carboxin from control experiment (Wegener medium under sterile conditions). Solvent system: chloroform-benzene 9 : 1 (double development).

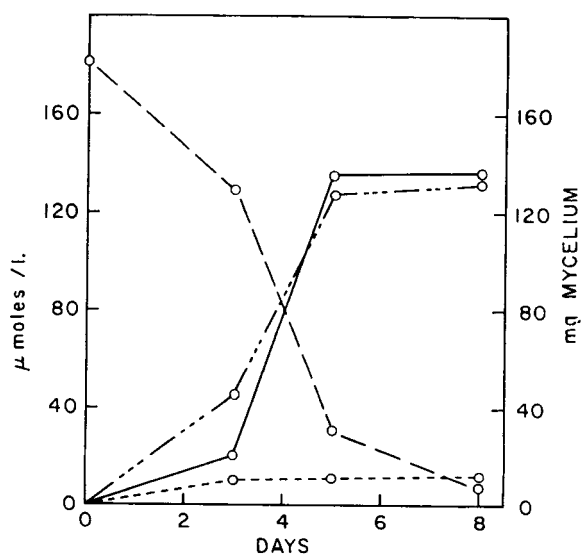


FIGURE 2 Degradation of Carboxin by *Rhizopus japonicus* in Wegener medium. ○—○ growth (dry weight of mycelium); ○---○ Carboxin; ○- - - -○ Carboxin sulfoxide; ○· · · · ·○ Oxycarboxin.

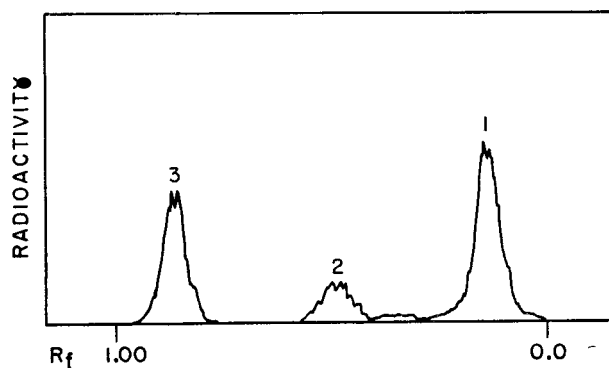


FIGURE 3 Radio-TLC scan of the chloroform extract of culture medium (steep) in which *Rhizopus japonicus* had been growing for 17 days in the presence of ^{14}C -labeled Carboxin. 1: Carboxin sulfoxide. 2: metabolite M-3. 3: Carboxin. Solvent systems, chloroform-benzene 9 : 1 (double development).

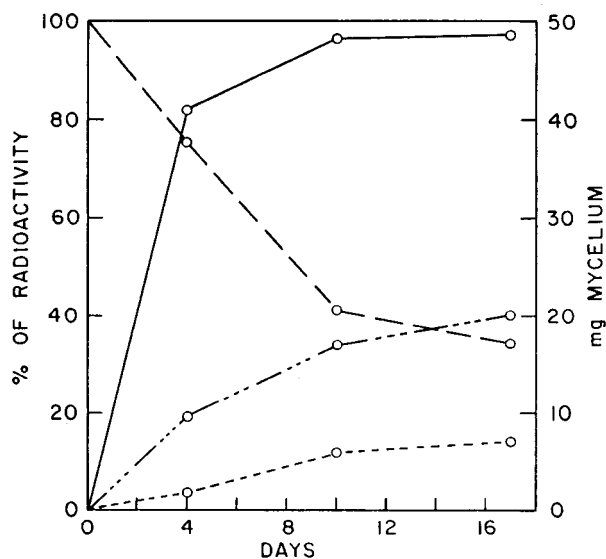


FIGURE 4 Degradation of Carboxin by *Rhizopus japonicus* in steep medium. ○—○ growth (dry weight of mycelium); ○— —○ Carboxin; ○— · · · · ·○ Carboxin sulfoxide; ○— · · · · ·○ Metabolite M-3.

in the growth medium. Carboxin, at a concentration of 160 $\mu\text{M}/\text{l}$ is completely converted to the two metabolites II and III which apparently are not further degraded by the fungus (Figures 1 and 2).

Metabolism of Carboxin under Limited Anaerobic Conditions

When *Rhizopus japonicus* is grown in steep medium without agitation, limited anaerobic conditions exist. This is shown e.g. by formation of ethanol, which is also observed in *Mucor* species when grown under limited oxygen supply. As in the Wegener medium, Carboxin sulfoxide (II) is the major metabolite but no Oxycarboxin is observed. Under these partial anaerobic conditions, however, a new metabolite M-3 is produced in fair yield. A metabolite with identical u.v. maximum has previously been observed under similar conditions and has been recognized as substituted anilide.¹⁴ Further details are shown in Figures 3 and 4.

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References

1. M. Snel, B. von Schmeling, and L. V. Edgington, *Phytopathology* **60**, 1164 (1970).
2. J. R. Hardison, *Phytopathology* **61**, 731 (1971).
3. B. von Schmeling and M. Kulka, *Science* **152**, 659 (1966).
4. W. T. Chin, G. M. Stone, and A. E. Smith, *J. Agr. Food Chem.* **18**, 709 (1970).
5. W. T. Chin, G. M. Stone, and A. E. Smith, *J. Agr. Food Chem.* **18**, 731 (1970).
6. M. Snel and L. V. Edgington, *Phytopathology* **60**, 1708 (1970).
7. W. T. Chin, G. M. Stone, and A. E. Smith, Proceedings of the Fifth British Insecticide and Fungicide Conference (1969), Vol. 2, p. 322.
8. W. T. Chin, G. M. Stone, and A. E. Smith, Presented at The International Symposium on Pesticide Terminal Residues (IUPAC), Tel Aviv, Israel (1971).
9. A. I. Allan and J. B. Sinclair, *Phytopathology* **59**, 1548 (1969).
10. P. Wallnöfer, M. Königer, S. Safe, and O. Hutzinger, *J. Agr. Food Chem.* **20**, 20 (1972).
11. P. Wallnöfer, S. Safe, and O. Hutzinger, *Pesticide Biochem. Physiol.*, in press.
12. R. Herber, J. Villoutreix, and M. Pierfitte, *C. R. Soc. Biol.* **163**, 241 (1969).
13. P. Wallnöfer and H. J. Rehm, *Zentrbl. Bakt.* **112**, 25 (1968).
14. P. Wallnöfer, *Naturwissenschaften* **55**, 351 (1968).