Metabolism of the Systemic Fungicide 2,5-Dimethyl-3-furancarboxylic Acid Anilide (BAS 3191) by *Rhizopus japonicus* and Related Fungi

Peter R. Wallnöfer,* Manfred Königer, Steve Safe,[1] and Otto Hutzinger[1]

Rhizopus japonicus and related fungi convert the systemic fungicide 2,3-dimethyl-3-furancarboxylic acid anilide (BAS 3191) into two metabolites, 2-hydroxymethyl-5-methyl-3-furancarboxylic acid anilide (M-2) and 2-methyl-5-hydroxymethyl-3-furancarboxylic acid anilide (M-1). These metabolites, which were identified by nuclear magnetic resonance and mass spectrometric analysis, accumulate after a 1 week growth period. BAS 3191 (60 μmol/l.) was transformed into 23 μmol/l. of M-1 and 12 μmol/l. of M-2, respectively.

A new fungicide for systemic control of barley and wheat loose smut is 2,5-dimethyl-3-furancarboxylic acid anilide (BAS 3191). Since it is useful as a seed treatment for food crops, a clear understanding of its fate in soil and crops is of interest. In this investigation the metabolism of BAS 3191 by pure cultures of certain fungi isolated from soil was studied. Since soil fungi of the genera *Phycomycetes* were previously shown to be very active in transforming anilide fungicides (Carboxin; Wallnöfer, 1968) all tests were performed with different strains of *Rhizopus* and *Mucor* in this study.

MATERIALS AND METHODS

Chemicals and Instrumentation. An analytical grade sample of BAS 3191 was obtained from a commercial formulation by purification with chloroform extraction followed by a cleaning procedure with alumina columns (activity grade V). The silica gel used for thin-layer chromatography (tlc) was silica gel 254 LSG, Schleicher & Schüll, Germany. Ultraviolet (uv) and nuclear magnetic resonance (nmr) spectra were recorded on Beckman DB and Varian A-60-A instruments, respectively. A Varian 6058 A spin decoupler was used in the decoupling experiments. The mass spectral data were obtained on a DuPont CEC 21-110 B mass spectrometer. A controlled temperature probe was used for introduction of the samples directly into the ion source.

The melting points were determined on a Kofler Hot Stage (Reichert, Austria). A Varian gas chromatograph Model 1800 equipped with a flame ionization detector and a 5 ft × 2 mm i.d. glass column packed with 1.5% XE 60 on Kieselguhr DMCS 70–100 mesh was used. Temperatures were 240° C for the column and 270° C for the injector and detector. Gas flow rates were 30 ml/min for the carrier gas nitrogen, 30 ml/min for hydrogen, and 60 ml/min for oxygen (minimum detectability limit of BAS 3191, M-1, M-2: 10 ng).

Culture Methods. The test organisms *Rhizopus japonicus*, *R. nigricans*, *R. peka*, and two strains of *Mucor* isolated from soil were grown in a synthetic glucose medium of Wegener *et al.* (1967) in 1-l. Fernbach vessels, containing 500 ml of culture medium on a shaker at 27° C. Calcium carbonate (1.5%) was added to the medium to neutralize the acid production by the fungi during the 1 week incubation period.

A standard solution of BAS 3191 (10 mg in 1 ml of acetone–propylene glycol = 1:1) was added to the culture medium to a final concentration of 25 mg/l.

Analytical Procedures. 10-ml portions of the culture medium were extracted with 30 ml of chloroform daily during the 1 week incubation period to observe the fungicide conversion. Aliquots were purified by tlc (silica gel, benzene–acetic acid 9:1) and BAS 3191 and its metabolites eluted with chloroform prior to quantitative determination by uv or glc analysis.

Isolation and Identification of BAS 3191 Metabolites. After removing the mycelial mass by filtration, the medium (500 ml) was extracted with 1000 ml of chloroform. Residual fungicide in the hyphae was extracted by Soxhlet extraction with methanol. The chloroform extract was dried (Na_2SO_4) and the volume reduced to about 0.5 ml by rotary evaporation. Chloroform extract from culture medium was streaked onto a preparative silica tlc plate (0.5 mm thickness), eluted with benzene–acetic acid (9:1), and the two bands corresponding to metabolite M-1 and metabolite M-2 were removed from the plate and the silica was extracted with chloroform. Both chloroform extracts were concentrated and further purified on alumina columns (activity grade V, 20 × 1.5 cm). By using chloroform as solvent and collecting 5-ml portions, the metabolites appeared between fraction 4 and 9. Fractions for M-1 and for M-2 were again streaked onto preparative tlc plates and eluted with chloroform–acetone–acetic acid, 15:2:3 (M-1 R_f = 0.70, M-2 R_f = 0.93). After elution of the corresponding bands, both metabolites were recrystallized from chloroform–carbon tetrachloride. The chemical structures (Figure 1) of the compounds were elucidated on the basis of physical evidence (Table I).

RESULTS AND DISCUSSION

Shortly after the beginning of the exponential phase of fungal growth, metabolites M-1 and M-2 could be observed

Bayerische Landesanstalt für Bodenkultur, Pflanzenbau und Pflanzenschutz, Munich, Germany.
[1] Atlantic Regional Laboratory, National Research Council of Canada, Halifax, Nova Scotia, Canada.

Table I. Physical Data for BAS 3191 and Metabolites

Compd	nmr Signals CH_3	nmr Signals CH_2OH	nmr Signals =C–H (Furan)	Mass spectra (intensity) M^+	*m/e* 93	*m/e* 123	*m/e* 139	mp, °C	tlc A^a R_f	tlc B^b R_f	glc retention time, min	uv λ_{nm}^{max}
BAS 3191	7.75 (d) (J = 1 cps) 7.42 (s)		3.90 (d) (J = 1 cps)	215 (40)	3	100		92–94	0.94	0.99	1.0	266
M-1	7.35 (s)	5.45 (s)	3.58 (s)	231 (45)	10	15	100	109–110.5	0.3	0.7	3.6	265
M-2	7.70 (d) (J = 1 cps)	5.22 (s)	3.77 (J = 1 cps)	231 (95)	90	15	100	112–113.5	0.7	0.93	4.8	268

[a] A = Benzene–acetic acid, 9:1. [b] B = Chloroform–acetone–acetic acid, 15:2:3.

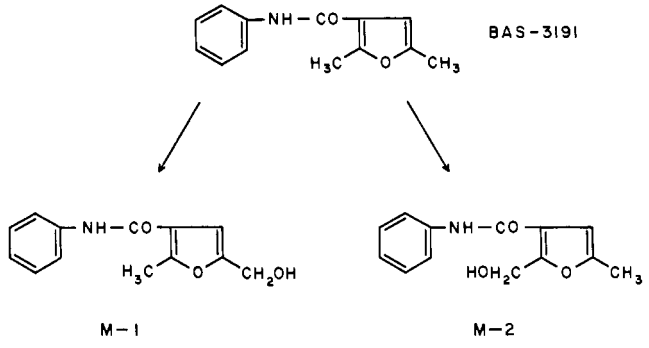

Figure 1. BAS 3191 (2,5-dimethyl-3-furancarboxylic acid anilide) and its conversion products M-1 (2-methyl-5-hydroxymethyl-3-furancarboxylic acid anilide) and M-2 (2-hydroxymethyl-5-methyl-3-furancarboxylic acid anilide)

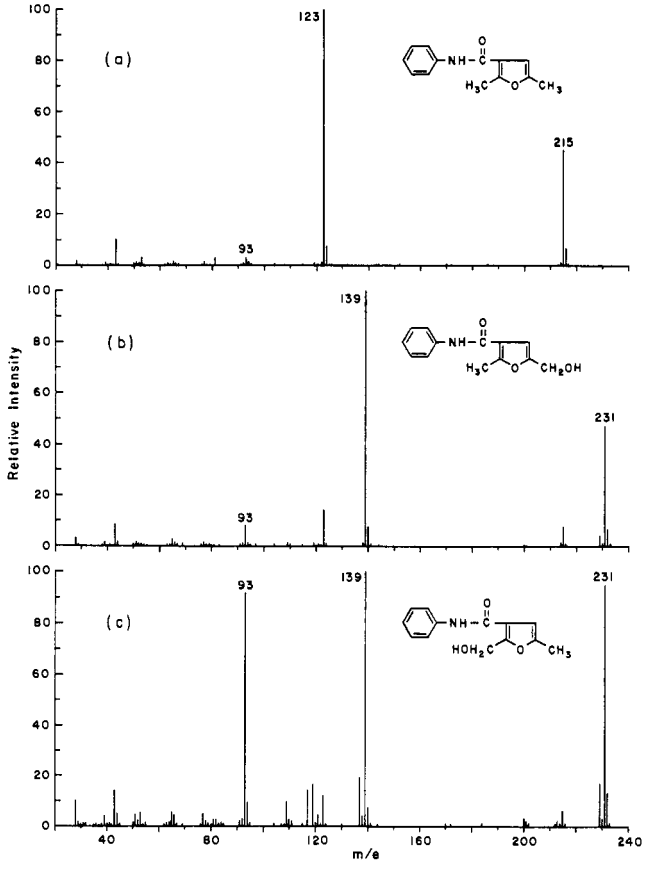

Figure 2. Mass spectra (70 eV) (sample temperature 80° C in each case): (a) BAS 3191; (b) M-1; (c) M-2

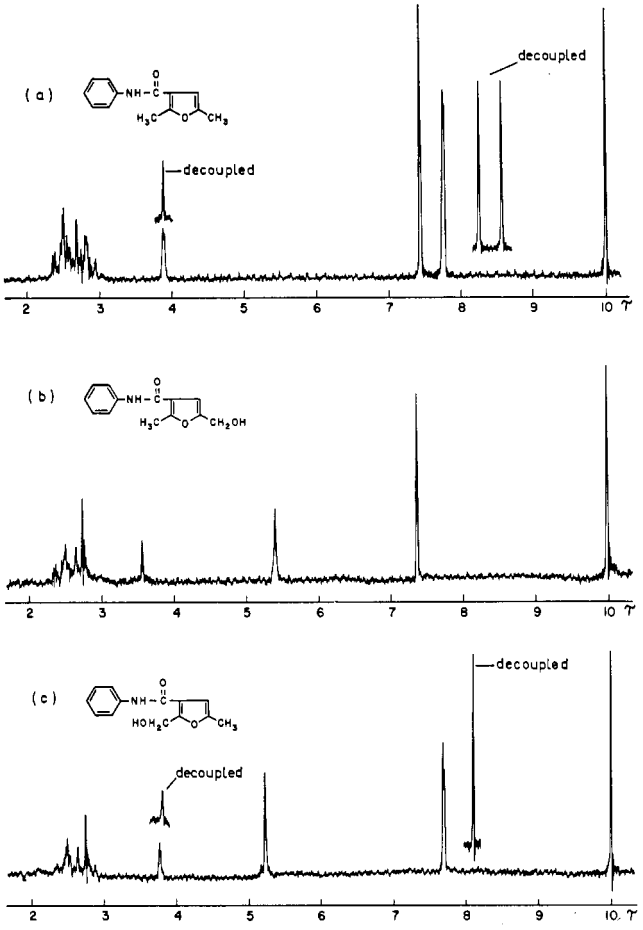

Figure 3. Nmr spectra of: (a) BAS 3191; (b) M-1; (c) M-2. Internal standard: tetramethylsilane

in the culture medium. The metabolites reached the highest concentration, when fungal growth ended and sporulation appeared. The metabolites were not being further degraded by the fungi. Within 1 week BAS 3191 was transformed, at the rate of 60 μmol/l. into 23 μmol/l. of M-1 and 12 μmol/l. of M-2. Unchanged fungicide (10–20%) was found in the mycelium of the fungi.

Identification of Metabolites. Both new metabolites (M-1 and M-2) gave mass spectra showing molecular ions at *m/e* 231, whereas 2,5-dimethyl-3-furancarboxylic acid anilide gives a strong molecular ion at *m/e* 215 (Figure 2), thus indicating possible introduction of a hydroxyl group into the two new metabolites. The mass spectrum of both M-1

and M-2 exhibited intense ions at *m/e* 139 with minor peaks at *m/e* 93. The mass spectrum of BAS 3191 showed cleavage between the amide nitrogen and carbonyl with hydrogen transfer to the nitrogen to give the substituted furan (*m/e* 123) and anilino (*m/e* 93) fragment ions. Thus the replacement of the *m/e* 123 peak in 2,5-dimethyl-3-furancarboxylic acid anilide by the *m/e* 139 ion in the two metabolites M-1 and M-2 indicated introduction of the hydroxyl substituent into the furan part of the molecule.

The nmr spectra of M-1, M-2, and fungicide BAS 3191 are shown in Figure 3. The two methyl groups of the fungicide are distinguished by a sharp singlet at τ 7.42 for the 2-methyl substituent, whereas the 5-methyl group appears as a doublet at τ 7.75 due to allylic coupling with the furan proton at C-4. The nmr spectrum of M-1 exhibits a singlet at τ 7.35 and a two proton signal at τ 5.45 typical of a methylene group carrying an hydroxyl substituent. These data are consistent with introduction of the hydroxyl on the 5-methyl substituent. The spectrum of M-2 exhibited a doublet at τ 7.70 (J = 1 Hz) due to allylic coupling of a methyl group with the low field furan proton. A signal at τ 5.22 (2 protons) is again typical of methylene group substituted with a hydroxyl substituent. These data are consistent with the hydroxylation of the 2-methyl group to give the polar metabolite M-2.

LITERATURE CITED

Wallnöfer, P., *Naturwissenschaften* **55,** 351 (1968).
Wegener, W. S., Schell, J. E., Romano, A. H., *J. Bacteriol.* **94,** 1951 (1967).

Received for review April 29, 1971. Accepted June 28, 1971. P. R. W. would like to thank the Federal German Ministry of Food, Agriculture and Forestry, and the Deutschen Forschungsgemeinschaft for financial support.