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N. Sethunathan[1]
Tomio Yoshida*

Department of Soil Microbiology
The International Rice Research Institute
Los Banos, Laguna, Philippines

[1] Central Rice Research Institute
Cuttack-6
Orissa, India

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Nature of Carboxin (Vitavax)-Derived Bound Residues in Barley Plants

More than 70% of the unextractable residue derived from [^{14}C]carboxin (5,6-dihydro-2-methyl-1,4-oxathiin-3-carboxanilide) in barley leaves was liberated by hot dimethyl sulfoxide. The ^{14}C residue so released was identified as carboxin (30%) and its sulfoxide (70%). The theory of lignin complex formation for detoxification through immobilization by plants is further indicated.

In a previous paper (Chin *et al.*, 1970), the possible existence of a highly insoluble complex of carboxin (5,6-dihydro-2-methyl-1,4-oxathiin-3-carboxanilide) with lignin was reported. Further identification, however, was not successful because of the relative instability of carboxin to hydrolysis. Hot dimethyl sulfoxide (DMSO) is known to be a good solvent for lignin (Weise and Niclas, 1967) and has been used in this work for releasing the insoluble [^{14}C]carboxin derived residues from barley plants. We also report here the results of our study of the nature of the DMSO-solubilized portion of the insoluble residue.

EXPERIMENTAL SECTION

Plant Materials. Two-hundred barley seeds were treated with hetero-ring-tagged [^{14}C]carboxin by applying an acetone solution of the chemical with a micropipette on each seed. Similarly, 200 seeds were treated with phenyl-tagged [^{14}C]carboxin and the same amount was left untreated. The seeds were treated at the rate of 3 oz of active ingredient per 100 lb of seed (corresponding to 0.25 μCi at 1 mCi/mM). Seeds were planted and grown in the greenhouse and the plants were harvested 30 days later. The first two leaves with the lower stem (about 1.5 in.) were combined, dried at 50°, ground, and extracted in a Soxhlet extractor for 8 hr with a mixture of benzene–acetone–methanol (1:1:1, v/v). The ^{14}C residues remaining in the extracted plant materials were considered to be insoluble and used for DMSO extraction.

DMSO Extraction. One-half gram of extracted plant material was stirred in a stoppered Erlenmeyer flask with a magnetic stirrer with 20 ml of DMSO for 4 days at room temperature and 5 hr at 80°. After cooling, the mixture was filtered through a coarse glass filter. The weight of plant material was reduced by this extraction to 0.3 g. A third extraction (hot) caused no significant reduction in tissue weight and removed only small amounts of ^{14}C.

Identification of Residues. After DMSO was evaporated to dryness at 85° and 15 mm, the ^{14}C-containing residue was taken up in methanol and analyzed by thin-layer chromatography on Eastman Kodak silica gel sheets No. 6061 in chloroform (A), acetone (B), and acetone–methanol (4:1, v/v) (C). For comparison, a DMSO plant extract obtained from untreated plants was fortified with labeled carboxin and its sulfoxide and used as reference standard. After development, the radioactive components were detected by autoradiography; the radioactive spots on the chromatogram were cut out and counted by liquid scintillation. The R_f values for carboxin and its sulfoxide were: in solvent A, 0.82 and 0.63; in solvent B, 0.88 and 0.70; and in solvent C, 0.91 and 0.84. In each solvent, complete resolution between components was obtained. To determine if the sulfoxide is not formed by chemical oxidation, a 1% solution of carboxin in DMSO was heated for 4 hr at 75–80°, as given in the extraction procedure. No detectable amounts of the sulfoxide were found when analyzed on thin layers in solvent A.

Determination of ^{14}C. All quantitative measurements of ^{14}C were made on the Beckmann LS-100 liquid scintillation spectrometer in a PPO-containing toluene–cellosolve based counting solution. The total radioactivity in plant tissue before and after DMSO extraction was determined by wet oxidation (Mahin and Lofberg, 1966) and liquid scintillation counting.

RESULTS AND DISCUSSION

By extraction of the ^{14}C residue-containing plant material with hot DMSO, about 74% of the total insoluble ^{14}C was solubilized for both ^{14}C-labeled substrates (Table I). Thin-layer chromatography showed that the DMSO-soluble part of ^{14}C residue in plants consists of two components. When compared with standard reference compounds in chloroform, acetone, and acetone–methanol, identical R_f values and shapes of spots as for carboxin and carboxin sulfoxide were obtained in each solvent. After autoradiography of the chromatograms, the two spots corresponding to carboxin and its sulfoxide were cut out and radioassayed by liquid scintillation counting. The ratio of carboxin to its sulfoxide was found to be 3:7 for both the phenyl and hetero-tagged compounds.

About 10% of the radioactivity from the DMSO extracts remained at the origin during thin-layer chromatography. When these radioactive areas were eluted from the gel and rechromatographed, carboxin and its sulfoxide were the principal compounds detected. They were present in the same ratios as in the original chromatogram. This indicated artificial retention by extraneous polar plant materials. A fortified sample from untreated plants gave similar results, confirming that the baseline spot was an artifact.

Table I. DMSO Extraction of Insoluble ^{14}C Residues from Barley Plants Grown from [^{14}C]Carboxin-Treated Seed

Extraction conditions	Hetero-ring-tagged, total 177,500 dpm/0.5 g		Phenyl-tagged, total 247,500 dpm/0.5 g	
	Insoluble	Soluble	Insoluble	Soluble
Room temperature, 4 days	96% (170,200 dpm)	2.3% (4000 dpm)	97% (240,000 dpm)	2.7% (6500 dpm)
2nd extraction, 80°, 5 hr	27% (4800 dpm)	70% (124,200 dpm)	33.2% (82,500 dpm)	67.5% (167,200 dpm)
3rd extraction, 80°, 5 hr	26% (45,645 dpm)	2% (3600 dpm)	28.1% (69,645 dpm)	4.0% (9000 dpm)

No other spots could be seen on the chromatograms. Under the conditions used for extraction with DMSO, carboxin was proven not to be oxidized chemically to the sulfoxide.

Aliquots of the DMSO extract from the phenyl-tagged tissues were steam distilled after caustic hydrolysis (Chin *et al.*, 1970) and the radioactivity originally in the extract was recovered almost quantitatively in the distillates. The steam-distilled radioactivity was probably aniline. This would further indicate that the ^{14}C residues were bound carboxin and its sulfoxide. While phenyl-ring hydroxylation has been noted in animals (Chin and Kucharczyk, 1972), these data show it did not occur in barley plants, because hydroxyanilines can not be steam distilled.

The nearly identical compositions of the ^{14}C-bound residues from barley plants treated with phenyl- and hetero-ring-tagged carboxin confirmed previous conclusions that hydrolysis of the amide bond of carboxin does not take place in barley plants. It is known that plants have the ability to absorb the sulfoxide derived from carboxin through the roots and also to convert carboxin to the sulfoxide within the plant (Chin *et al.*, 1970). For this reason, the detection of a greater amount of the sulfoxide in bound residues is not surprising. The presence of carboxin in bound residues indicates that binding takes place quickly after this fungicide is absorbed from the soil. The recovery of the unchanged carboxin and the absence of derivatives other than the sulfoxide proves that these bound residues are not the result of extensive plant metabolism, but rather involve complex formation. Similar effects have been reported for other anilides (Chin *et al.*, 1964). The gradual release of carboxin and its sulfoxide by increasingly severe extraction conditions and the fact that 26–28% of ^{14}C is still in the remaining undissolved plant material support the theory of detoxication of the original compound or its sulfoxide by immobilization. The sulfoxide of carboxin is the only metabolite which we have detected in plants.

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Wei-Tsung Chin
Norbert Kucharczyk*
Allen E. Smith

Uniroyal Chemical
Division of Uniroyal, Inc.
Naugatuck, Connecticut 06770

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