potential biological activity of the different metabolites.

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Position of the Radiolabel in Glycine Resulting from [2-^{14}C]DPX-3217 (^{14}C-Labeled Curzate Fungicide) Metabolism

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Studies using *Peptococcus glycinophilus*, a glycine-specific bacterium, demonstrated that radiolabeled glycine in plants treated with [2-^{14}C]DPX-3217 (^{14}C-labeled Curzate fungicide) was exclusively labeled in the methylene group, corresponding to the position in the parent compound. These findings provide evidence that glycine is a primary metabolite of DPX-3217 rather than a product of metabolic reincorporation of the radiolabeled carbon.

The active ingredient in Du Pont Curzate fungicide, 2-cyano-*N*-[(ethylamino)carbonyl]-2-(methoxyimino)-acetamide (DPX-3217), is an effective fungicide against grape downy mildew as well as tomato and potato late blight. In studies on tomatoes, potatoes, and grapes reported by Belasco et al. (1980), ^{14}C-labeled glycine was found to be a primary metabolite of [2-^{14}C]DPX-3217. Similarly, Belasco and Baude (1980) found [^{14}C]glycine to be the major metabolite in rats dosed with [2-^{14}C]DPX-3217. The relatively high concentrations of radiolabeled glycine in crops and in the urine of rats treated with DPX-3217 and the structural relationship of DPX-3217 suggested that glycine might be a direct metabolite of DPX-3217. This study was undertaken to demonstrate that the radiolabeled glycine isolated from both potato and tomato plants treated with [2-^{14}C]DPX-3217 was present primarily as [2-^{14}C]glycine, using an anaerobic glycine-specific bacterium, *Peptococcus glycinophilus*.

EXPERIMENTAL SECTION

Radiolabeled Chemicals. [1-^{14}C]-Glycine and [2-^{14}C]glycine were purchased from Amersham/Searle Corp., Arlington Heights, IL 60005. Each compound had a radiochemical purity of 95% and a specific radioactivity of 40 mCi/mmol. [2-^{14}C]DPX-3217 was synthesized according to the procedure described by Belasco and Baude (1980). This preparation had a radiochemical purity of >99% and a specific activity of 1.90 mCi/mmol or 9.57 μCi/mg.

Bacterium. *P. glycinophilus* (ATCC 23195) was obtained from the American Type Culture Collection, Rockville, MD 20852. In the earlier literature, this organism also was known as *Deplococcus glycinophilus*.

Culture Media. Stock cultures of *P. glycinophilus*, an anaerobic bacterium, were carried in Bacto-Thioglycollate medium without dextrose (Difco Laboratories, Detroit, MI). This culture medium was supplemented with 0.3 g of glycine per L of medium to support the growth of this glycine-specific bacterium.

An experimental nutrient medium described by Cardon and Baker (1947) was also used. This medium was supplemented with sterile sodium sulfide (0.01% concentration) after autoclaving as a means of maintaining anaerobic conditions.

In those tests employing washed cell suspensions of *P. glycinophilus*, a minimal medium containing 0.04 M glycine, 0.02 M phosphate buffer, pH 7.0, and 0.02% $Na_2S{\cdot}9H_2O$ was used. Here, also, sterile sodium sulfide was added after the medium containing the other ingredients was autoclaved.

Source of DPX-3217 Metabolites. (1) Potato tubers were lyophilized from plants which had received five weekly foliar applications of [2-^{14}C]DPX-3217 at a rate equivalent to 0.2 kg/(1000 L ha) (Belasco et al., 1980).

(2) Tomato fruit was lyophilized from plants which had received seven weekly foliar treatments of [^{14}C]DPX-3217 at a concentration of 0.15 kg/(1000 L ha) (Belasco and Baude, 1980).

The lyophilized plant tissues were hydrolyzed in 6 N hydrochloric acid under reflux (100 °C) for 24 h. The hydrolysate was filtered and the residue washed with water. The filtrate was reduced to near dryness on a vacuum rotary evaporator and redissolved in 5 mL of water. The pH was adjusted to 7.0, and the solution was again taken to near dryness. This concentrate was then picked up in a small volume of water (0.5–1.0 mL) for microbiological assay.

Microbiological Procedure. Twenty milliliters of the culture medium was introduced into a 50-mL screw-cap Erlenmeyer flask which had a center well containing 1 mL of 10% sodium hydroxide solution to absorb metabolic $^{14}CO_2$ for radioassay. The culture medium was then supplemented with the ^{14}C-labeled test substrate, e.g., ^{14}C-labeled glycine or radiolabeled tissue hydrolysate. The

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Table I. Metabolism of [2-^{14}C]- and [1-^{14}C]Glycine by *P. glycinophilus*

^{14}C-labeled substrate	nutrient medium	[1,2-^{14}C]CH_3COOH		$^{14}CO_2$	
		dpm[a] × 10^{-3}	%	dpm[a] × 10^{-3}	%
[2-^{14}C]glycine	Cardon and Baker	1021.0	99.3	7.2	0.7
	minimal	154.0	96.4	5.8	3.6
	Bacto-Thioglycollate	111.7	94.8	6.1	5.1
[1-^{14}C]glycine	minimal	3.6	8.3	39.8	91.7
	Bacto-Thioglycollate	6.6	3.6	179.1	96.4

[a] dpm = disintegrations per minute, a measure of radioactivity.

Table II. Metabolism of ^{14}C-Labeled Glycine in Plant Tissue Hydrolysates

^{14}C-labeled substrate	nutrient medium	[1,2-^{14}C]CH_3COOH		$^{14}CO_2$	
		dpm × 10^{-3}	%	dpm × 10^{-3}	%
potato tuber hydrolysate	Bacto-Thioglycollate	157.3	93.9	10.3	6.1
potato stem hydrolysate	Bacto-Thioglycollate	24.4	91.4	2.3	8.6
tomato fruit hydrolysate	Bacto-Thioglycollate	62.4	92.7	4.9	7.3

system was autoclaved at 15 psig for 15 min followed by the addition of sterile sodium sulfide solution, when using the Cardon and Baker or the minimal nutrient media. Each flask was inoculated with 1 mL of a concentrated washed cell suspension from a 16–24-h culture of *P. glycinophilus*. The flasks and contents were then incubated at 37 °C for 48 h before analysis.

Sample Preparation and Analysis. Following incubation, the caustic in the center well of the flask was removed and radioassayed in a Searle Isocap 300 scintillation counter before and after treatment with an excess of a saturated aqueous barium chloride solution to determine whether the absorbed radioactivity was due to $^{14}CO_2$ and/or other volatile radioactive materials. The fermentation mixture in the flask was removed and adjusted to pH 10 with 1 N sodium hydroxide. The solution was taken to dryness on a vacuum rotary evaporator at 45 °C. The dry residue was suspended in 0.5–1.0 mL of water and the pH adjusted to 1 with 2 N hydrochloric acid. The resultant slurry was diluted to 2.0 mL and centrifuged to remove bacterial cell debris and other solids prior to radioassay by scintillation counting and radio-gas chromatographic (GC) analysis for radiolabeled acetic acid. The samples were chromatographed by using a Model 810 F&M gas chromatograph with a flame ionization detector, equipped with a 4:1 splitter. The major fraction of the column effluent was channeled into vials containing scintillation solution (Formula 947, New England Nuclear Corp.) to permit simultaneous radioassay of the various volatile fractions of the injected sample as indicated by the recorder response. A 2 m long glass column (7 mm o.d. × 4 mm i.d.) packed with Poropak Q was used for the gas chromatographic separation. With the column temperature programmed at 6 °C/min from 100 to 225 °C and a flow rate of 60 mL of He/min, the retention time for acetic acid was 11 min. The [^{14}C]acetic acid in the experimental samples was confirmed by using a Du Pont mass spectrometer, Model 21-492, coupled to a Perkin-Elmer gas chromatograph, Model 990 (GC/MS).

RESULTS AND DISCUSSION

Experimental data (Baker et al., 1948; Cardon and Baker, 1947; Sagers and Gunsalus, 1961) show that *P. glycinophilus* has the unique ability to ferment glycine, and not the other common amino acids, to acetic acid, ammonia, and carbon dioxide. Glycine radiolabeled in the methylene carbon yields [^{14}C]acetic acid, while that labeled in the carbonyl carbon yields $^{14}CO_2$ almost exclusively (Figure 1). Consequently, glycine labeled in both carbon atoms will yield both [^{14}C]acetic acid and $^{14}CO_2$.

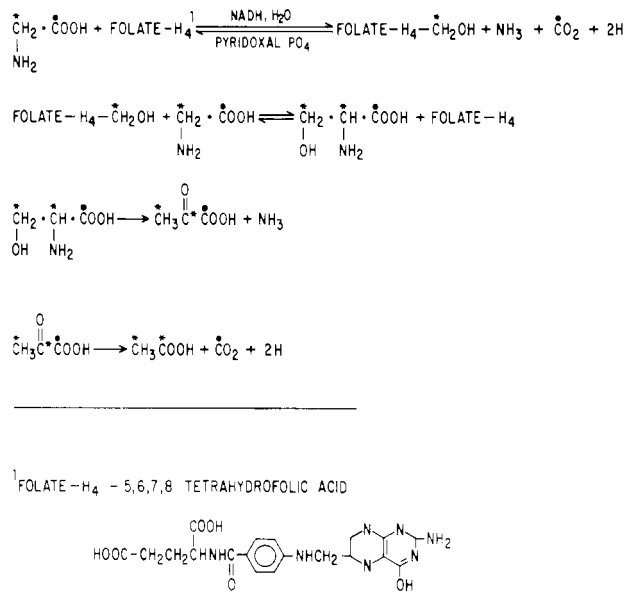

Figure 1. Biochemical conversion of labeled glycine to acetic acid by *P. glycinophilus* (Sagers and Gunsalus, 1961). Symbols represent labeling in either the 1 (●) or 2 (*) position.

Preliminary evaluation of *P. glycinophilus* metabolism of both [1-^{14}C]- and [2-^{14}C]glycine in the various nutrient media confirmed the findings reported by Baker et al. (1948) and Sagers and Gunsalus (1961). Greater than 90% of the radioactivity was present as $^{14}CO_2$ when [1-^{14}C]-glycine was the radiolabeled substrate. Conversely, the acetic acid formed in the anaerobic decomposition of [2-^{14}C]glycine contained more than 95% of the radiolabel (Table I).

Replacement of the [^{14}C]glycine in the nutrient medium with acid hydrolysates of tomato fruit, potato tuber, and potato stem resulted in the formation of radiolabeled acetic acid which accounted for more than 90% of the total radioactivity. Radiolabeled CO_2 accounted for less than 10% (Table II) of the total. On this basis, it appears evident that glycine is a primary metabolite of DPX-3217 rather than a metabolic sink for reincorporated carbon.

The above conclusion appears consistent with the previous findings (Belasco et al., 1980; Belasco and Baude, 1980) in which radiolabeled glycine was found to be a major biodegradation product in crops and in rats treated with ^{14}C-labeled DPX-3217. The high level of [^{14}C]glycine found in relation to the total radioactivity plus the structural relationship of DPX-3217 and its metabolites (Belasco et al., 1980) to glycine lends credence to the

concept that glycine is a direct metabolite of DPX-3217 rather than a naturally occurring compound in which the radiolabel has been metabolically incorporated.

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Stereochemical Effects in Carbamate Insecticides: Effect of *E,Z* Configuration on the Insecticide Activity of 1,1-Bis(methylthio)-3,3-dimethyl-2-butanone *O*-[(Alkylamino)carbonyl]oximes

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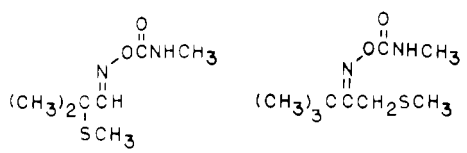
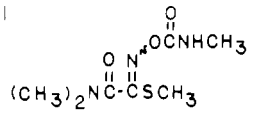
A series of 1,1-bis(methylthio)-3,3-dimethyl-2-butanone *O*-[(alkylamino)carbonyl]oximes and the *S*-oxides of 1,1-bis(methylthio)-3,3-dimethyl-2-butanone *O*-[(methylamino)carbonyl]oxime were synthesized. The *E* and *Z* isomers of the oximes were separated and their absolute stereochemistry established by use of the Beckmann rearrangement. The carbamates were found to be effective insecticides having activity against the Mexican bean beetle (*Epilachna varivestis*), two-spotted spider mite (*Tetranychus urticae*), and Black Bean aphid (*Aphis fabae*). The *Z* isomers were consistently more active than the *E* isomers. In vitro studies indicated the compounds to be cholinesterase inhibitors.

Since the early 1950s many structural variations of oxime carbamates have been made and reported as effective insecticides. Although the *E–Z* isomerism (also called syn–anti) that results from the stereochemical integrity of the oxime functionality has been recognized as an important structural feature of this class of compounds as well as carbamoylated thiohydroximidates, reported comparisons of the insecticidal activity of such isomers are few. For example, the aldoxime, aldicarb (1), was proposed as the single *E* isomer and its insecticidal activity reported



E aldicarb
1

Z thiofanox
2



oxamyl
3

(Payne et al., 1966). The insecticidal data for the *Z* isomer was not given. An undefined single configurational isomer of the ketoxime thiofanox (2) was more recently reported to have superior insecticidal activity over a 1:1 mixture of isomers (Magee and Limpel, 1977). Although the two isomers of oxamyl (3) have been prepared, their absolute configurations and their biological activities were not reported (Buchanan, 1971).

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The *E* and *Z* isomers of isophorone *O*-[(methylamino)-carbonyl]oxime have been prepared and differences in the insecticidal activity reported to be insignificant (Figure 1) (Metcalf and Fukuto, 1967). A similar result was reported for the isomeric pair of *exo*-3-chloro-*endo*-6-cyano-2-norbornanone *O*-[(methylamino)carbonyl]oximes (Figure 2) (Payne et al., 1967). The insecticidal activity and configurational assignments of the related thiohydroximidate carbamate, methomyl (4), have been carefully examined. The configuration of the *E* and *Z* isomers of methomyl (4) were determined via Beckmann rearrangement (Davies et al., 1968) and later confirmed by X-ray crystallography (Waile and Sim, 1971).

The insecticidal activity of the *Z* isomer was found to be significantly and consistently (over several species of insects) greater than that of the *E* isomer (Figure 3). A 40-fold increase of the *Z* over the *E* isomer on the Vetch aphid was the greatest difference noted. Consistent with the potent activity of these compounds, the *Z* isomer was 100 times more effective in inhibiting flyhead cholinesterase.

The *Z* isomer of the corresponding oxygen analogue of methomyl, methyl *N*-[(methylamino)carbonyl]acetimidate, was reported to have greater activity than the *E* isomer (Felton, 1968). This is consistent with the report by Donninger et al. (1968) on the activity of a series of oxygen analogs.

From the limited number of reported comparisons of insecticidal data, it appears that the isomeric nature of the oxime is important for activity in the thio and oxy hydroximidate type compounds and has limited effect on carbamates of ald- and ketoximes. This is unusual in that both types of compounds are reported to be cholinesterase inhibitors and presumably have a similar, if not identical, mode of action. One might expect the isomerism to be important since acetylcholinesterases are known to be highly stereospecific in the hydrolysis of the optical isomers

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