Fate of Furadan (NIA-10242) in Bean Plants

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The metabolism of Furadan^{R} (2,3-dihydro-2,2-dimethyl-7-benzofuranyl N-methylcarbamate) in animals has been investigated and the metabolic pathway largely defined (1). Both hydrolytic and oxidative mechanisms were involved in the alteration of this insecticide by rats and house flies. The hydrolytic product 2,3-dihydro-2,2-dimethyl-7-hydroxybenzofuran was detected in the free and conjugated form, although its concentration was much greater as a water-soluble conjugate. Oxidation of Furadan yielded 2,3-dihydro-2,2-dimethyl-3-hydroxybenzofuranyl-7-N-methyl-carbamate which was conjugated directly or metabolized to the 3-keto-carbamate. The hydrolysis products of each of these carbamate metabolites were isolated from the treated animals.

Radiotracer techniques were used in the current study of the metabolic fate of Furadan in plants. Young bean plants were injected with the carbamate and the radioactive residues evaluated as to their identity and relative magnitudes.

METHODS

Furadan-carbonyl-C^{14} (specific activity 2.0 mc/mM) and Furadan-ring-C^{14} (specific activity 0.21 mc/mM) were used in this

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study. The appropriate amount of radiolabeled Furadan was dissolved in a 2:1 water-acetone solution and 50 ul of this mixture injected into the stem of young bean plants (Garden Snapbeans, Contender Variety). Each plant received 6.7 ug of Furadan-carbonyl-C^{14} or 200 ug of Furadan-ring-C^{14}. The rather large quantity of the latter was necessary because of its low specific activity. For analysis, each plant was cut into small pieces and thoroughly homogenized in an acetone-water mixture. The residue was again extracted by homogenation in chloroform. After the necessary filtering and partitioning, the radiolabeled materials in the plant were divided into 3 separate fractions. The first fraction (organo-extractables) contained those radioactive residues which partitioned into chloroform, the second fraction (water-solubles) consisted of radioactivity which remained in the water layer, and the third fraction (unextractables) included those residues which could not be extracted from the plant solids. The amount of radioactivity in both liquid phases was determined by liquid scintillation counting of 0.2 ml aliquots. Radioactive residues in the plant solids were measured by oxygen combustion of 1 gram samples of the dried plant material (2).

The organo-extractable fraction of the plant homogenate was prepared for analysis using thin layer chromatography (TLC)

by concentrating the solvent and removing the plant pigments by coagulation. Three milliliters of acetone and 20 ml of coagulating solution (3) were added to the residue after the solvent was evaporated. The flask was then held at room temperature, with occasional shaking, for 15 minutes. The mixture was then filtered and the residue washed several times with small volumes of coagulating solution. The filtrate was extracted 3 times with chloroform, and the extract concentrated and applied to silica gel G TLC plates. All chromatograms were developed 2 dimensionally with 3:1 ether-hexane as the first solvent system and 4:1 methylene chloride-acetonitrile as the second solvent system. Radioactive areas on the chromatograms were detected by radioautography.

For metabolite identification, each radiolabeled material was extracted from TLC and its cochromatography with standards (Table I) determined using several solvent systems previously reported (1). Non-labeled Furadan and metabolite standards were located by spraying the plates with potassium hydroxide and a fluoborate spray reagent (4). The materials appeared as red spots.

To evaluate the nature of the carbonyl-C^{14} plant-water-solubles, the water layer was concentrated to 0.5 ml and applied as a band to a TLC plate prepared 0.5 mm thick. The TLC was developed in a 5:3:2 ethyl acetate:n-propanol:water mixture. Two radioactive bands; Band B (R_f 0.3) and Band C (R_f 0.8) were

TABLE I

Chemical Identity and R_f Values of Furadan and Its Metabolites Detected in Bean Plants

Chemical Name	R_f [2]	Abbreviation
Unknown	0.00	Unknown I
Unknown	.10	Unknown II
2,3-dihydro-2,2-dimethyl-3-hydroxybenzofuranyl-7-N-hydroxymethylcarbamate	.19	3-OH-N-CH_2OH-Furadan (3-OH-N-CH_2OH) [3]
2,3-dihydro-2,2-dimethyl-3-hydroxy-benzofuranyl-7-N-methylcarbamate [1]	.37	3-OH-Furadan
Unknown	.40	Unknown III
2,3-dihydro-2,2-dimethyl-3-Keto benzofuranyl-7-N-methylcarbamate [1]	.48	3-Keto-Furadan
2,3-dihydro-2,2-dimethyl-7-benzofuranyl-N-methylcarbamate [1]	.54	Furadan
2,3-dihydro-2,2-dimethyl-3,7-dihydroxybenzofuran [1]	.61	3-OH-Furadan-phenol (3-OH-phenol)
2,3-dihydro-2,2-dimethyl-3-Keto-7-hydroxybenzofuran [1]	.86	3-Keto-Furadan-phenol (3-Keto-phenol)
2,3-dihydro-2,2-dimethyl-7-hydroxybenzofuran [1]	.90	Furadan phenol (phenol)

[1] Authenic samples supplied by FMC Corp., Niagara Chemical Division, Middleport, New York.

[2] Silica gel G chromatograms developed in 3:1 ether-hexane.

[3] Abbreviations in parenthesis are used in tables to designate the metabolites.

separated with this solvent system. Each band was extracted from the gel with methanol, the methanol evaporated and 2 ml of water added to the tubes containing the residues. Two milliliters of 1 N HCl were added and the tubes placed in a boiling water bath for 10 minutes. Radioactive products converted to organo-extractables by the acid treatment were extracted into ethyl acetate. This extract was analyzed by TLC as described above. The ring-C^{14}, plant-water-solubles were similarly analyzed except that the TLC step prior to the acid treatment was omitted because of the low amount of radioactivity present.

RESULTS

Furadan-carbonyl-C^{14}, 3-OH-Furadan-ring-C^{14} and Furadan-Phenol-ring-C^{14} were added individually to control bean plants and their recovery and stability using the procedures described above determined. Both of the carbamate compounds were recovered in quantities exceeding 95 percent of the added radioactivity. However, approximately 25 percent of the Furadan-phenol was lost as the extracting solvents were concentrated for application to TLC. Further loss of the phenol-ring-C^{14} was observed during the exposure of the chromatogram to x-ray film. By allowing the solvents to evaporate very slowly and by spraying the developed TLC with a dilute potassium hydroxide solution prior to exposure to film, the recovery of the phenol was increased to 85-90 percent. The compounds were not altered chemically by any of the procedures used for analyzing the bean

extracts.

The metabolism of Furadan-carbonyl-C^{14} by bean plants is shown in Table 2. The plant was very effective in altering the injected carbamate. Only 32 percent of the dose remained as Furadan after 3 days and less than 1 percent after 14 days. Total recovery of the injected radioactivity from the 3-day plants was 71 percent, indicating that some hydrolysis of the carbamate, with loss of carbon-14 dioxide, had occurred. The percentage recovery from subsequent sample was only slightly less and was paralleled by an increase in the amount of water-soluble Furadan equivalents. Apparently, these latter metabolic products were not rapidly degraded in the plant.

The identity of the Furadan-contributing portion of the water-soluble residues are designated in Table 2 by the letters B and C after the name of the metabolite. These letters identify those products which, as water-soluble metabolites, were separated into 2 components by TLC. The "B" metabolites were freed by acid treatment of Band B (R_f 0.3) and the "C" metabolites from identical treatment of Band C (R_f 0.8). Since the same Furadan metabolites, except Unknown III, were recovered from each band of conjugates (Table 2), the existance of at least 2 naturally occurring chemicals capable of conjugation with the metabolites was evident.

Identification of the major metabolic products of Furadan in plants showed that the metabolic pathway was the same as reported in animals (1). In both cases, the metabolite of greatest

TABLE 2

Nature and Magnitude of Furadan and Metabolites in Bean Plants Injected with Furadan-Carbonyl-C^{14}.

Metabolites	Percent of Total Furadan Injected (Days)					
	3	5	7	14	21	28
Furadan A [1]	31.6	15.5	8.1	2.3	0.7	0.4
3-OH-Furadan A [1]	7.9	3.5	3.6	1.0	1.8	1.9
3-OH-Furadan B [1]	2.6	4.2	6.0	6.1	7.0	9.3
3-OH-Furadan C [1]	18.6	31.0	32.5	31.5	26.7	26.2
3-Keto-Furadan A	7.9	3.5	3.6	1.0	1.8	1.9
3-Keto-Furadan B	.8	.7	1.4	2.4	3.5	4.6
3-Keto-Furadan C	.1	.3	.3	1.3	3.4	3.7
3-OH-N-CH_2OH A	.1	.1	.1	0	0	0
3-OH-N-CH_2OH B	.1	.2	.3	2.2	1.3	.3
3-OH-N-CH_2OH C	.2	.4	.2	.2	.3	.4
Unknown I A	.1	.1	.1	.1	.2	.8
Unknown I B	.7	1.2	1.9	1.3	2.9	1.2
Unknown I C	.6	1.3	2.1	.9	.8	.9
Unknown III C	1.3	2.6	3.0	4.7	3.6	2.5
Unknown IV [2] B	1.5	.5	.2	.1	.2	.9
Unknown IV B	1.9	4.8	5.1	3.3	2.9	2.3
Unextractables	2.6	3.4	3.5	3.8	3.1	2.3
Total Recovery	70.9	70.2	68.8	61.7	57.9	57.7

[1] Metabolites followed by the letter A were extractable from the plant with organic solvent. B=radioactive band (R_f 0.3) of water soluble conjugates as separated on TLC, extracted and treated with acid to free the carbamate moiety. C=radioactive band (R_f 0.8) of water soluble conjugates treated the same as B.

[2] Radioactivity in the water-soluble portion of the plant homogenate that would not extract into ethyl acetate after acid treatment.

magnitude was 3-OH-Furadan. This material was observed in plants as the free carbamate, but never in amounts equal to its concentration as a water-soluble conjugate. Other metabolites identified in both the free and conjugated form were 3-keto-Furadan and 3-OH-N-CH_2OH-Furadan. Of the unknown metabolites detected, it is likely that only Unknown III was a single component. The R_f of this product in the 3:1 ether-hexane system was 0.4, whereas Unknown I was that radioactivity which remained at the origin on TLC. Unknown IV includes all the radioactive components of the water-soluble portion of the plant homogenate that would not extract into ethyl acetate after acid treatment.

Results obtained using the Furadan-carbonyl-C^{14} were substantiated by identical experiments using Furadan-ring-C^{14} (Table 3). With the radiocarbon in this position, it was possible to more clearly define the hydrolytic pathway indicated in the first experiment. Furadan-ring-C^{14} was degraded slower than the carbonyl-C^{14} -labeled dose probably because of the large amount of actual Furadan, 200 ug, injected into each plant. The carbamate metabolites and their relative magnitudes were the same as observed in the first treatment. In addition, the hydrolytic products Furadan-phenol, 3-OH-Furadan-phenol and 3-Keto-Furadan-phenol were isolated from bean plants treated with Furadan-ring-C^{14} (Table 3). Only Furadan-phenol was found in the free form and its concentration was lower than that detected as a water-soluble conjugate. Recovery of the ring-C^{14} -treatment from plants

TABLE 3

Nature and Magnitude of Radioactive Residues in Bean Plants Injected with Furadan-Ring-C^{14}.

Metabolites	Percent of Total Furadan-C^{14} Injected (Days)					
	3	5	7	14	21	28
Furadan A [1/]	58.7	54.6	26.5	18.2	11.4	13.6
3-OH-Furadan A [1/]	11.1	8.3	8.6	2.1	1.6	1.2
3-OH-Furadan B [1/]	16.2	25.7	33.8	49.1	48.5	35.5
3-Keto-Furadan A	.1	.4	.6	.4	.1	.2
3-Keto-Furadan B	.9	1.1	4.5	2.9	2.2	2.6
3-OH-N-CH_2OH A	.1	.2	.1	0	0	0
3-OH-N-CH_2OH B	.2	.1	.1	.1	.2	.2
Phenol A	.2	.2	.1	0	0	0
Phenol B	.2	.4	.7	.7	1.0	1.0
3-OH-Phenol B	.3	.6	.9	1.8	1.8	2.1
3-Keto-Phenol B	.2	.5	.8	.4	.6	.4
Unknown I A	.1	.2	.1	.2	.2	.2
Unknown I B	1.3	.8	.9	1.0	.8	1.1
Unknown II B	.3	.1	.3	.4	.5	.2
Unknown III B	.7	.6	1.3	2.2	2.1	1.6
Unknown IV B [2/]	1.5	1.5	4.3	6.9	5.0	5.8
Unextractables	3.4	3.3	6.5	7.1	9.3	9.8
Total Recovery	95.5	98.6	90.1	93.5	85.3	75.5

[1/] Metabolites followed by the letter A were extractable from the plant with organic solvent. B=metabolites freed from water-soluble conjugates by acid hydrolysis.

[2/] Radioactivity in the water-soluble portion of the plant homogenate that would not extract into ethyl acetate after acid treatment.

analyzed within 14 days after treatment was greater than 90 percent. However, there was a gradual decrease in the percentage recovery from plants harvested at 21 and 28 days.

The nature of the Furadan metabolites detected in bean plants suggests that hydrolysis, oxidation and conjugation are important pathways in the metabolism of this carbamate. From a standpoint of residue analysis, it is obvious that an analytical procedure should be capable of detecting the parent compound and at least 3 carbamate metabolites. The actual residual importance of these Furadan metabolites can be evaluated much more realistically after a practical analytical method has been developed and residue experiments are conducted under field conditions.

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