Metabolism of 2,2-Dimethyl-2,3-dihydrobenzofuranyl-7 *N*-Methylcarbamate (Furadan) in Plants, Insects, and Mammals

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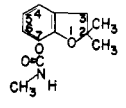
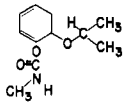
The systemic insecticide Furadan is metabolized by hydroxylation and hydrolysis in plants, insects, and the mouse. The key metabolites are produced by hydroxylation at the benzylic carbon to give 3-hydroxy-Furadan, which is subsequently oxidized to the 3-keto-Furadan when not blocked by formation of conjugates. The 3-keto-Furadan is hydrolytically unstable, and has an intense blue fluorescence which is characteristic of the 2,2-dimethylbenzofuran-3-one structure. Metabolites which were identified with certainty were 3-hydroxy-Furadan and 3-keto-Furadan and their respective 7-hydroxy hydrolysis products. These phenols, together with Furadan phenol, were present in the free state, and were also identified as conjugates, principally the glucosides, in the various biological systems. There is also evidence of the formation of trace amounts of *N*-CH_2OH derivatives in insect and mammal produced from Furadan or its 3-OH or 3-C=O derivatives.

The compound, 2,2-dimethyl-2,3-dihydrobenzofuranyl-7 *N*-methylcarbamate (Furadan, Niagara 10242), is a new systemic insecticide with broad spectrum activity. In standardized laboratory tests, 400 mg. of a 5% granular formulation of Furadan, applied uniformly about the base of cotton plants, approximately 2 feet high in 6-inch pots, produced 80 to 100% mortality of the following species caged in the upper leaves for the indicated number of weeks after treatment: cotton aphid, *Aphis gossypii*, 9 weeks; cotton leaf perforator, *Bucculatrix thurberiella*, 13 weeks; and salt marsh caterpillar, *Estigmene acrea*, 9 weeks. The compound was, however, ineffective against the cotton mite, *Tetranychus cinnabarinus*. In field tests applied to cotton as a 10% granular at 3 pounds per acre in early July, Furadan gave virtually seasonal protection from attacks of leafhoppers, lygus bug nymphs, and other noxious species, and resulted in yield increases up to 400 pounds of lint cotton per acre (Reynolds, 1967).

Furadan and some of its derivatives are highly active contact insecticides, as shown in Table I. This insecticidal activity and anticholinesterase action of Furadan are of theoretical interest because of the structural resemblance of Furadan to the insecticide 2-isopropoxyphenyl *N*-methylcarbamate (Baygon). Thus, in Furadan, the isopropoxy anionic interactant is fixed in position by the fused dimethylfuran ring (Metcalf and Fukuto, 1965).



FURADAN



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The practical usage of Furadan as a systemic insecticide depends upon a knowledge of its metabolic fate in plants and animals. This paper describes its metabolism in cotton and corn plants, in the housefly, *Musca domestica*, and the salt marsh caterpillar larva, *Estigmene acrea*. Preliminary investigations have also been made of the urinary metabolites in the white mouse.

MATERIALS AND METHODS

Three radiotracer preparations of Furadan were used. The synthesis of each of these is described as follows.

4,5,6-H^3-2,2-Dimethyl-2,3-dihydrobenzofuranyl-7 *N*-Methylcarbamate. To a mixture of 1.0 gram of ring-labeled H^3-catechol (Nuclear-Chicago) and 0.5 gram of sodium methoxide in 5 ml. of methanol was added 0.82 gram of methallyl chloride. The solution was stirred and heated at reflux temperature for 18 hours. The methanol was removed in a rotatory evaporator and the product, 2-methallyloxyphenol, was taken up in chloroform, washed three times with water, and dried over sodium sulfate. Distillation gave 0.64 gram of the mono-

Table I. Biological Properties of Furadan and Related Carbamates

Compound	Fly CHE, $I_{50}M$	*Musca domestica*, Topical LD_{50}, μg. per G.	*Culex pipiens*, LC_{50}, P.P.M.
Furadan	2.5×10^{-7}	6.7	0.054
3-OH Furadan	1.4×10^{-6}	>500	0.75
3-C=O Furadan	1.3×10^{-5}	>500	1.7
N-CH_2OH Furadan	2.9×10^{-5}	>500	5.2
3-C=O, *N*-CH_2OH Furadan	1.3×10^{-3}	>500	>10
3-$OC(O)CH_3$ Furadan	1.0×10^{-6}	>500	0.8
3-OCH_3 Furadan	6.4×10^{-7}	40	0.34
5-Cl Furadan	1.0×10^{-7}	500	0.37
4-Cl Furadan	2.8×10^{-7}	45	0.182
4-CH_3 Furadan	1.6×10^{-7}	3.9	0.145
Baygon	6.9×10^{-7}	25.5	0.30

methallyl ether (b.p. 74° to 78° C., 0.5 mm.). Heating the 2-methallyloxyphenol at 235° C. for 30 minutes gave 0.48 gram of 7-hydroxy-2,2-dimethyl-2,3-dihydrobenzofuran (b.p. 72° to 76° C., 0.3 mm.). Treatment of the distillate with 0.17 gram of methyl isocyanate and a trace of triethylamine produced, after recrystallization from equal parts of methanol and water, 0.35 gram of 4,5,6-H^3-2,2-dimethyl-2,3-dihydrobenzofuranyl-7 *N*-methylcarbamate (H^3-Furadan) (m.p. 149° to 152° C.). The final product had a radiopurity of 99+% as determined by thin-layer chromatography (TLC), and a specific activity of 1.68 mc. per mmole.

2,2-Dimethyl-2,3-dihydrobenzofuranyl-7 *N*-$C^{14}H_3$-Carbamate. *N*-$C^{14}H_3$ methylisocyanate (0.1 gram), prepared according to Bartley *et al.* (1966), reacted in the usual manner with 0.28 gram of 7-hydroxy-2,2-dimethyl-2,3-dihydrobenzofuran in the presence of a trace of triethylamine. The product had a radiopurity of 99+% as determined by TLC, and a specific activity 0.104 mc. per mmole.

C^{14} aromatic ring-labeled (position 7a) Furadan was obtained from the Niagara Chemical Division, FMC Corp. This compound was synthesized from ring-labeled phenol as the starting material, and had a radiochemical purity of 99+% with a specific activity of 0.22 mc. per mmole.

SYNTHESIS OF MODEL METABOLITES

The dimethyldihydrobenzofuran nucleus affords a number of potential pathways for oxidative metabolism. The following compounds were synthesized as model metabolites for comparison with those produced in plants and animals.

7-Hydroxy-2,2-dimethyl-2,3-dihydrobenzofuran (Furadan phenol) was obtained by hydrolysis of Furadan carbamate (Niagara Chemical Division, FMC Corp.) in alcoholic potassium hydroxide, acidification, and extraction in ether to give a colorless liquid (b.p. 63° to 65° C., 0.1 mm., n_D^{25} 1.5455). This material also was obtained according to the procedure described above for the preparation of tritium-labeled Furadan phenol.

2,2-Dimethyl-3-acetoxy-2,3-dihydrobenzofuranyl-7 *N*-methylcarbamate (3-acetoxy Furadan) was prepared by refluxing a solution of Furadan in 10 to 1 glacial acetic acid and acetic anhydride with a fourfold molar excess of potassium permanganate. After 5 hours, the mixture was diluted with water, and sodium sulfite added to reduce the manganese dioxide to soluble salts. Further dilution precipitated 3-acetoxy Furadan which was recrystallized from methanol (m.p. 156° to 158° C.: per cent calculated for $C_{14}H_{17}NO_5$, C = 60.20, H = 6.14; found C = 60.86, H = 6.10). Nuclear magnetic resonance spectrum was in accord with the assigned structure showing the following proton resonance: a narrow doublet centered at 8.55 (nonequivalent *gem*-di-CH_3), singlet at τ 7.93 (acetyl CH_3), doublet centered at τ 7.13 (*N*-CH_3), singlet at τ 6.07 (3-H), and a multiplet centered near τ 2.9 (aromatic H).

2,2-Dimethyl-3-hydroxy-2,3-dihydrobenzofuranyl-7 *N*-methylcarbamate (3-hydroxy-Furadan) was obtained in almost quantitative yield by hydrolysis, at reflux, of 3-acetoxy Furadan in dilute sulfuric acid. The product, m.p. 138° to 140° C., crystallized out of the aqueous solution upon cooling: per cent calculated for $C_{12}H_{15}NO_4$, C = 61.01, H = 5.97; found, C = 60.48, H = 6.52. NMR spectrum showed the following proton resonance: a doublet centered at 8.60 (nonequivalent *gem*-di-CH_3), singlet at τ 7.61 (3-OH), doublet centered at τ 7.13 (*N*-CH_3), singlet at τ 5.30 (3-H), and a multiplet centered at about τ 2.9 (aromatic H). The infrared spectrum is shown in Figure 1.

2,2-Dimethyl-3-keto-2,3-dihydrobenzofuranyl-7 *N*-methylcarbamate (3-keto-Furadan) was prepared by reaction of Furadan in glacial acetic acid with a fourfold molar excess of chromium trioxide at 25° to 35° C. for 16 hours, diluting with water, and extracting the product with ether. Removal of the ether, and recrystallization from ethanol gave 3-keto-Furadan (m.p. 187° to 188° C.: per cent calculated for $C_{12}H_{13}NO_4$, C = 62.54, H = 5.52; found, C = 62.08, H = 6.17). NMR spectrum showed a singlet at τ 8.54 (*gem*-di-CH_3), doublet centered at τ 7.09 (*N*-CH_3), and a multiplet centered near τ2.74 (aromatic H). The infrared spectrum of 3-keto-Furadan is shown in Figure 1.

2,2-Dimethyl-7-hydroxy-3-keto-2,3-dihydrobenzofuran, (3-keto-Furadan phenol) was prepared by hydrolysis of 3-keto-Furadan according to the procedure described above for the hydrolysis of Furadan. Recrystallization from water gave yellow needles (m.p. 159° to 165° C: per cent calculated for $C_{10}H_{10}O_3$, C = 66.66, H = 5.59; found, C = 66.84, H = 5.72).

3-Keto-6-hydroxy-2,3-dihydrobenzofuranyl-7 *N*-methylcarbamate was prepared as a model metabolite to evaluate the possible effects of hydroxylation of the aromatic ring, after a number of failures to prepare the corresponding 2,2-dimethyl analog. 3-Keto-6,7-dihydroxy-2,3-dihydrobenzofuran (6,7-dihydroxycoumar-3-one) was prepared by the method of Feurstein and Brose (1904), and the 6,7-bis-*N*-methylcarbamate by reaction with two equivalents of methylisocyanate in dry ether with a trace of triethylamine catalyst. Repeated recrystallization from methyl ethyl ketone gave the 6,7-bis-*N*-methylcarbamate (m.p. 178° to 180° C.: per cent calculated for $C_{12}H_{12}N_2O_6$, C = 51.43, H = 4.31; found, C = 51.83, H = 4.53). This compound was dissolved at room temperature in one equivalent of 0.1*M* aqueous sodium hydroxide. Acidification with hydrochloric acid gave a single compound believed to be 3-keto-6-hydroxy-2,3-dihydrobenzofuranyl-7 *N*-methylcarbamate, which when recrystallized from methyl ether ketone melted with decomposition at 185° to 187° C.: per cent calculated for $C_{10}H_9NO_5$, C = 53.81, H = 4.06; found C = 53.78, H = 3.99. NMR in deuteriodimethyl sulfoxide showed the following proton resonance: two doublets centered at τ 2.60 and τ 3.26 for two nonequivalent aromatic H each mutually split by 9.0 cps, a singlet at τ 5.27 (2-H), a broad peak centered near τ 6.38 (aromatic OH), and a doublet centered at τ 7.42 (*N*-CH_3). The infrared spectrum is shown in Figure 1. The position of the carbamoyl moiety was not established unequivocally (6 or 7 *N*-methylcarbamoyloxy), and our assignment of the compound as the 7-carbamoyloxy derivative is based on the higher reactivity of the 6-carbamoyloxy moiety predictable on mesomeric grounds.

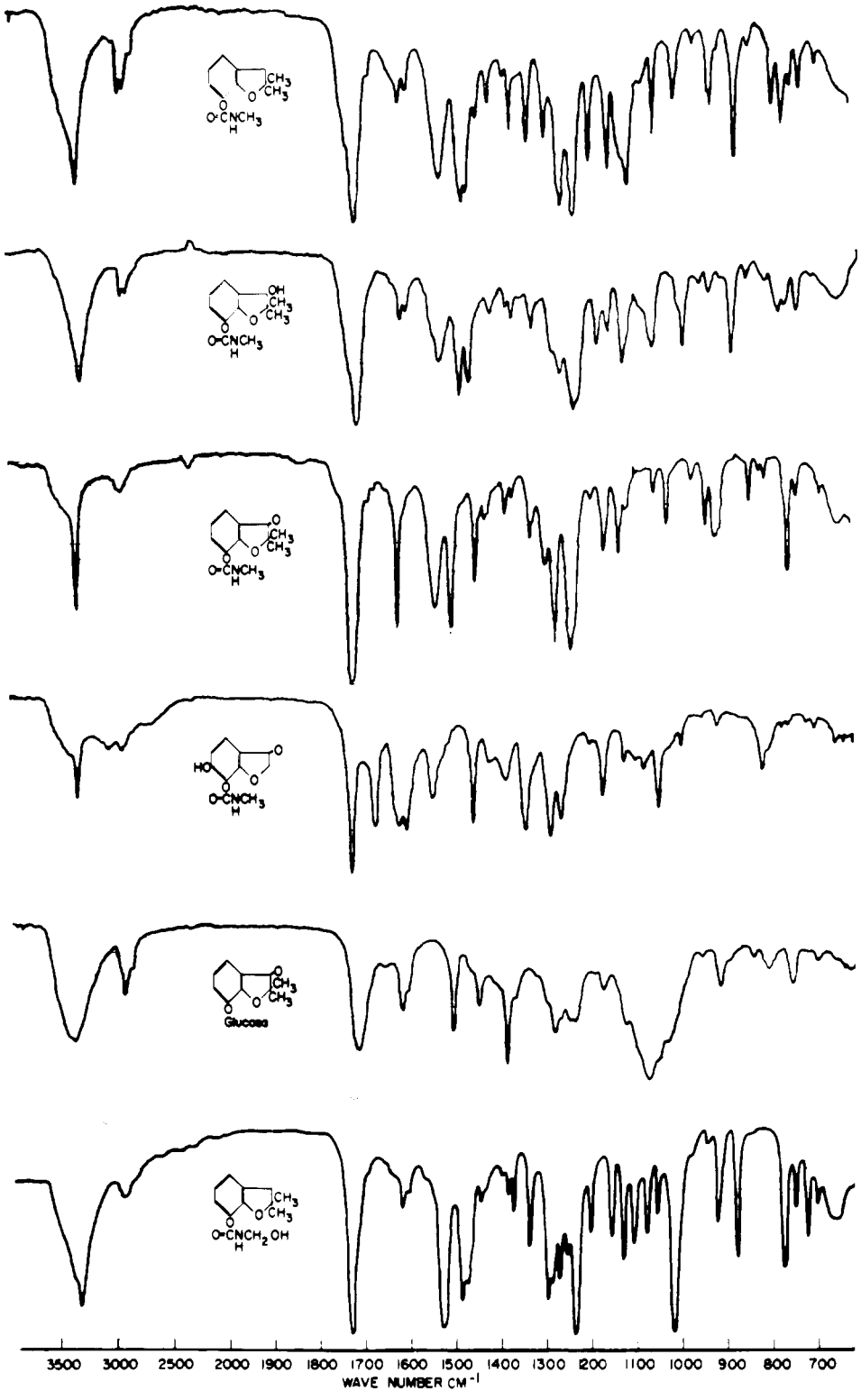


Figure 1. Infrared spectra of Furadan and its principal metabolites

2,2-Dimethyl-2,3-dihydrobenzofuranyl-7 *N*-hydroxymethylcarbamate was prepared by hydrogenation of the corresponding *N*-methylbenzyloxycarbamate prepared by reaction of benzyloxymethyl isocyanate with Furadan phenol in a pressure bottle with a few drops of triethylamine catalyst. The carbamate was extracted with a larger volume of diethyl ether, and recrystallized from ether-hexane (m.p. 54° to 56° C.: per cent calculated for $C_{19}H_{21}NO_4$, C = 69.72, H = 6.42; found, C = 69.89, H = 6.66). The *N*-benzyloxy Furadan (0.5 gram or 0.0016 mole) was dissolved in 5 ml. of equal parts of dry 2-propanol and ethanol, and added to a hydrogenation bottle containing 1.5 gram of 10% palladium on charcoal saturated with 18.4 ml. of H_2 for 20 minutes in 20 ml. of

2-propanol. Hydrogenation was continued until the theoretical amount (34.2 ml.) was consumed in about 1.5 hours. After filtration and removal of solvent under vacuum, the 2,2-dimethyl-2,3-dihydrobenzofuranyl-7 *N*-hydroxymethylcarbamate was crystallized in almost quantitative yield from ether-hexane 2 to 1 (m.p. 131 to 133° C.: per cent calculated for $C_{12}H_{15}NO_4$, C = 60.76, H = 6.33; found, C = 60.70, H = 6.30). The infrared spectrum (Figure 1) was consistent with the assigned structure, and NMR in deuteriochloroform showed proton resonance attributable to the aliphatic OH moiety at τ 7.82. The compound gave an unmistakable, violet chromotropic acid reaction in trace amounts consistent with the conversion of the *N*-CH_2OH group to formaldehyde. Its biological and chromatographic properties are given in Tables I and II.

2,2-Dimethyl-2,3-dihydro-3-ketobenzofuranyl-7 *N*-hydroxymethylcarbamate (3-C=O, *N*-CH_2OH Furadan), (m.p. 186 to 188° C.) was prepared exactly as above using 2,2-dimethyl-2,3-dihydro-3-keto-7-hydroxybenzofuran as the starting phenol. The compound (per cent calculated for $C_{12}H_{13}NO_5$, C = 57.37, H = 5.18; found, C = 57.13, H = 5.46) had a bright blue fluorescence in ultraviolet indicating the benzofuranone moiety and gave a greenish coloration with chromotropic acid indicating the *N*-CH_2OH group. Its infrared spectrum closely resembled that of *N*-CH_2OH Furadan, shown in Figure 1, with absorption at 1015 $cm.^{-1}$(s) attributable to the C—O stretching frequency of primary alcohols. This peak was much reduced and shifted toward 1050 $cm.^{-1}$ in the *N*-$CH_2OCH_2C_6H_5$ derivatives. The infrared spectrum of 3-C=O, *N*-CH_2OH Furadan showed a double peak at 1710 and 1730 for the two keto groups which was not observed in the KBr spectra of 3-C=O Furadan (Figure 1). The biological and chromatographic properties of this compound are given in Tables I and II.

The benzyloxymethyl isocyanate (b.p. 128° to 130° C. at 25 mm.) was prepared as described by Balba (1967) from benzyloxyacetyl chloride (b.p. 127° to 130° C. at 13 mm.) by reaction with activated sodium azide in the conventional manner.

The NMR spectrum of Furadan showed a singlet τ 8.52 (*gem*-di-CH_3), doublet centered at τ 7.18 (*N*-CH_3), singlet at τ 6.98 (3-H), a broad peak centered at τ 4.76 (N—H), and a multiplet centered at τ 3.10 (aromatic H). Because of the broadness of the N—H resonance, detection of this peak was difficult, and for this reason N—H absorptions are not reported for the compounds synthesized as model metabolites.

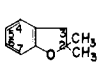
All NMR spectra were obtained with a Varian Model A-60 spectrometer in deuteriochloroform, except in a single case where solubility necessitated the use of deuteriodimethylsulfoxide. Tetramethylsilane was used as an internal standard.

CHROMATOGRAPHIC SEPARATIONS

The preliminary separations of radiolabeled metabolites were carried out as described by Metcalf *et al.* (1966) using chromatographic columns 2 × 30 cm. packed with 60- to 100-mesh silicic acid (Florisil) from a slurry in hexane. Leaf tissues or insect feces were homogenized in five times their weight of 70% ethanol, or acetone, concentrated to about 0.5 ml. in vacuo, and added directly to the top of the column. The chromatogram was developed by passing the following sequence of solvents through the column, and collecting individual 22-ml. fractions: Ether-hexane, 3 to 1, 330 ml.; chloroform, 220 ml.; ethyl acetate, 330 ml.; and methanol, 440 ml. All solvents were reagent grade or were distilled through a 1-meter column before use.

Thin-layer chromatography (TLC) was carried out in the usual manner using silicic acid (Absorbosil 1) 0.25 mm. thick, and the chromatograms were developed in one of the following mixtures: ether-hexane, 3 to 1 (EH); ether-benzene, 3 to 1 (EB); and butanol-ethanol-water, 10:2:3 (BEW). The chromatograms were evaluated by spraying with *p*-nitrobenzenediazonium fluoroborate, which produced a reddish color with the free hydroxy compounds, or after treatment with methanolic sodium hydroxide, with the hydrolyzed carbamates; 1% ninhydrin in pyri-

Table II. Properties of Benzofuranyl Carbamates and Derivatives



	M.P., ° C.	Elution from Column	Thin-Layer Chromatography,[a] R_f Values EH 3 to 1	EH 1 to 1	EB 3 to 1	BEW 10:2:3	Limit Detection, μg. Fluoroborate		Fluorescence	
7-OCONHCH$_3$	147	Ether-hexane-chloroform	0.36	0.18	0.50	0.72	Pink	0.1	None	>100
7-OH	B.p. 66–70 at 0.4 mm.	Ether-hexane	0.71	0.62	0.70	0.76	Pink[b]	0.3	None	
3-OH, 7-OCONHCH$_3$	138–140	Ethyl acetate	0.13	0.04	0.26	0.77	Yellow	1.0	Faint blue	30
3-OH, 7-OH		Ether-hexane	0.49				Pink[b]			
3-C=O, 7-OCONHCH$_3$	187–8	Ether-hexane	0.35	0.15	0.15	0.71	Pink	0.2	Blue-violet	0.01
3-C=O, 7-OH	159–165	Ethyl acetate	0.59	0.37	0.70		Pink	0.1	Blue	0.01
7-OCONHCH$_2$OH	133–5		0.21	0.0	0.22	0.72	Pink		None	
3-C=O, 7-OCONHCH$_2$OH	186–8		0.12		0.21		Pink		Violet	
3-OCOCH$_3$, 7-OCONHCH$_3$	156–8		0.36	0.13	0.54	0.73	Pink	3	Purple	10
3-OCH$_3$, 7-OCONHCH$_3$	105–9		0.75	0.44	0.54	0.73	Pink	0.1	Blue	3
5-Cl, 7-OCONHCH$_3$	157–160		0.45	0.24	0.61	0.75	Pink		Blue	1

[a] Systems EH = ether, hexane.
EB = ether, benzene.
BEW = butanol, ethanol, water.
[b] Coloration with fluoroborate alone, without NaOH.

dine with development at 100° C. for 30 minutes to form red spots with amines produced from the carbamyl group; and with sulfuric acid and aqueous sodium 1,8-dihydroxy-naphthalene-3,6-disulfonate (chromotropic acid) to give a violet to blue color with formaldehyde produced from the hydrolysis of the N-CH_2OH group.

The R_f values for the pure model compounds in the several solvent systems are given in Table II as the averages of at least three replicates. Despite standardization of thickness and moisture content of the TLC plates, the R_f values in individual runs varied somewhat, and in the presence of plant and animal extracts were generally slightly lower than those for the pure substances. Standard model compounds were run together with the unknowns in the metabolism experiments, and the values given in the text and in Figure 3 are those actually observed in various individual experiments.

The cotton plants were variety Delta-Pine Smoothleaf, and the corn plants Robson, Seneca 285. They were grown in the glasshouse at 80° F.

HYDROLYSIS OF CARBAMATE ESTERS

The relative hydrolytic stabilities of several metabolic products of Furadan directly affect the metabolic pathways in plant and animal tissues. Hydrolysis constants, K_{hyd}, were measured by adding the carbamates at 0.1% w./v. in methanol to phosphate buffer pH 9.5, and determining the rate of the appearance of the phenolic hydrolysis products by ultraviolet spectrophotometry with a constant temperature cell at 37.5°, giving the values shown in Table III. These illustrate the hydrolytic instability of 3-keto-Furadan and of N-CH_2OH Furadan which hydrolyzed approximately 170 and 12.5 times more rapidly, respectively, than the parent carbamate Furadan.

Table III. Hydrolysis of Furadan and Primary Metabolites in Phosphate Buffer, pH 9.5 at 37.5° C.

	Absorbance, Mμ	K_{hyd}, Min.$^{-1}$	$T^1/_2$, Min.
Furadan	241	0.0104	66.9
3-OH Furadan	300	0.0263	26.4
3-C=O Furadan	378	1.715	0.404
N-CH_2OH Furadan	241	0.130	5.33
3-C=O, N-CH_2OH Furadan	378	>7.0	ca. 0.1

OXIDATION WITH MODEL SYSTEMS

Udenfriend *et al.* (1954) and Brodie *et al.* (1954) have devised a model system for hydroxylation involving molecular oxygen and metal catalysis. This was applied to Furadan, 3-OH Furadan, and 3-C=O Furadan, adding 0.6 to 6.5 mmoles of EDTA, 1.4 mmoles of ascorbic acid, and 0.13 mmoles of $FeSO_4 \cdot 7H_2O$ in 25 ml. of 0.1M phosphate buffer at pH 6.6, and oxygenating for 16 hours at 37.5° C.

Organic reaction products were extracted with an equal volume of ether, concentrated, and examined by TLC with EH solvent. The results of such an experiment incorporating 1.2 mg. of C^{14}-ring labeled Furadan are shown in Table IV. The experiment shows the vulnerability of the benzylic carbon of Furadan to attack by the ·OH radical (Staudinger *et al.*, 1965), and suggests a logical parallel with cellular oxidations catalyzed by mixed function oxidases. The formation of the 3-OH and 3-C=O Furadan derivatives is also shown below to be the dominant metabolic pathway in living systems. The blue-white fluorescent (F) product R_f 0.10–0.16 (EH) appears from infrared spectra to be 2,2-dimethyl-2,3-dihydro-3-keto-6-hydroxybenzofuranyl-7 N-methylcarbamate (XII). This compound gave a positive ninhydrin reaction indicating the presence of the carbamate group, and upon hydrolysis with sodium hydroxide it produced a green color with ferric chloride, typical of o-dihydroxybenzenes (Reio, 1958).

Oxidation of 3-OH Furadan in the model system produced the 3-C=O Furadan, 3-C=O Furadan phenol, and 3-OH Furadan phenol together with the blue-white F derivative at R_f 0.16. Oxidation of 3-C=O Furadan produced the 3-C=O Furadan phenol, the blue-white product (R_f 0.13), its hydrolysis product (R_f 0.70), and unidentified products R_f 0.06 and R_f 0.80 which gave a red coloration with fluoroborate (FB).

METABOLISM IN VITRO BY MIXED FUNCTION OXIDASES

The in vitro metabolism of carbamates by mixed function oxidases has been effective in elucidating possible pathways of metabolism (Leeling and Casida, 1966). Microsomal oxidases from the housefly have been utilized with the radiolabeled Furadan. A typical experiment involved homogenization of housefly tissues combined with three times their weight of sucrose, and centrifuged to 50,000 × G to give the soluble plus microsomal fractions. The soluble and precipitated enzymes were made up in

Table IV. Oxidation of C-^{14}Ring Labeled Furadan by Udenfriend Reaction

R_f-EH, 3 to 1	C.P.M.	Per Cent	Reactions[a]	Probable Identity
0.0	1051	1.4		
0.08	899	1.2	Blue white F	3-C=O, 6-OH Furadan
0.16	9560	12.5	FB	3-OH Furadan
0.31	4476	5.8	Violet F	Unknown
0.37	4829	6.3	Violet F	3-C=O Furadan
0.43	49924	65.3	FB	Furadan
0.58	534	0.7	Blue F	3-C=O Furadan phenol
0.69	1677	2.2	Dark pink FB	
0.76	1680	2.2	FB	Furadan phenol

[a] F = fluorescence.
FB = diazonium fluoroborate.

6 ml. of phosphate buffer pH 7.0, containing 1 mg. of NADP, 5 mg. of glucose 6-phosphate, and 5 mg. of carbamate. Glucose 6-phosphate dehydrogenase is present in sufficient quantity in the fly homogenate to form NADPH. After incubation with O_2 for 2 hours at 37° C., the aqueous solution was extracted with ether, and the protein precipitated with acetone. The ether extract and the water residue were subjected to TLC with EH 3 to 1 on silicic acid. Thirteen distinct radiolabeled products were found as shown in Table V, and were partially characterized with chromogenic reagents, by fluorescence, and by comparison with known compounds.

The results of this experiment suggest attack by the ·OH radical at four sites on the Furadan molecule—the 3-position of the furan ring, the 6-position of the aryl ring, the *N*-CH_3 group, and the 2-methyl groups. These results, especially the formation of the *N*-CH_2OH derivatives, are in accord with in vitro results secured by Leeling and Casida (1966) through incubation of carbaryl with rat liver microsomes. From radioisotope experiments discussed elsewhere in this paper, it does not seem likely that the intact plant, insect, or mammal produces substantial amounts of metabolites with altered *N*-CH_3 or 2-CH_3 groups.

METABOLISM IN ISOLATED COTTON LEAVES

More than 20 experiments were conducted using 0.5 to 2 mg. of C^{14}-ring, *N*-$C^{14}H_3$-, and H^3-ring labeled Furadan imbibed into isolated cotton leaves with approximately 2 ml. of water containing a few drops of acetone. The leaves were homogenized, extracted, and subjected to column chromatography at intervals of 1, 2, 4, 6, 8, and 12 days after holding them at 80° ± 2° F. In these experiments, the total recovery of radioactivity in the organic solvents from the column ranged from 80 to 94% of that in the leaf homogenates.

The results of the column chromatography (Figure 2) show, in general, with all three radiolabeled products an increase over 8 days in the amount of polar compounds eluting in ethyl acetate and methanol. These polar metabolites are obviously forming in the leaf from the parent carbamate which is of low polarity and elutes at the ether-hexane-chloroform interface. This behavior is characteristic of that observed with other carbamates which are metabolized by oxidation and hydrolysis (Dorough and Casida, 1964; Metcalf *et al.*, 1966).

As an example of the variety of metabolic products formed in the intact cotton leaf, the following experiment is typical. Four days after imbibation of a large dosage of 2 mg. of H^3-labeled Furadan, column chromatography gave: ether-hexane eluate, 82%; ethyl acetate, 3.1%; and methanol, 14.9% of total eluted H^3. In a similar experiment with 2 mg. of C^{14}-ring labeled Furadan, the fractions eluted were: ether-hexane, 42.8%; ethyl acetate, 3.7%; and methanol, 47.6%. Two-dimensional TLC of the leaf extract gave the pattern shown in Figure 3. The compounds identified with certainty in this and the other similar experiments were as follows.

Parent Carbamate—Furadan (I) was present in ether-hexane eluate R_f 0.36 in EH, bright red color by fluoroborate after NaOH hydrolysis. The parent carbamate was recovered in relatively large amounts (>80% of total) in the early stages of the isolated cotton leaf experiments, but the amounts decreased rapidly, and little or none was found after 8 days.

Furadan Phenol or 2,2-dimethyl-7-hydroxy-2,3-dihydrobenzofuran (IV) elutes in ether-hexane, R_f 0.71 in EH, and gives an immediate bright red color with FB. Only small amounts of this phenol were found at 2 and 4 days. The identity of this compound was confirmed by finding identical FB-positive spots which cochromatographed with the synthetic model in the ether-hexane eluate from leaves treated with ring H^3 and *N*-$C^{14}H_3$ carbamates, but coincident radioactivity only with the tritium label.

3-OH Furadan or 2,2-dimethyl-3-hydroxy-2,3-dihydrobenzofuranyl-7 *N*-methylcarbamate (II) elutes in ethyl acetate and has R_f 0.13 in TLC with EH, is nonfluorescent, and gives a characteristic yellow color when treated with FB alone. The identity of this spot was confirmed by oxidation with CrO_3 in acetic acid to give the bright violet F (fluorescence) 3-C=O derivative and by TLC cochromatography of the radiolabeled spot R_f 0.13 with the synthetic model in EH, EB, and BEW solvents.

Table V. Metabolism of C^{14}-Ring Labeled Furadan by Mixed Function Oxidases of the Housefly

R_f–EH, 3 to 1	% C.P.M., Ether	% C.P.M., Aqueous	Reactions[a]	Probable Identity
0.0	0.0	1.2	FB, F, N	3-C=O, di-OH Furadan
0.10	0.0	0.5	FB, F, N	3-C=O, 6-OH Furadan
0.16	0.0	3.6	FB, N	3-OH Furadan
0.25	0.0	3.8	FB, N	
0.33	0.1	0.2	FB, F, N	3-C=O Furadan
0.38	7.2	0.0	FB, N	Furadan
0.44	38.1	1.5	FB, F, CA, N	2-CH_2OH, 3-C=O Furadan
0.48	24.5	1.7	FB, F, CA, N	3-OH, *N*-CH_2OH Furadan
0.53	14.1	0.4	FB, F	3-C=O Furadan phenol
0.65	1.0	0.5	FB	Furadan phenol
0.70	1.2	0.0	FB, F	
0.75	0.4	0.0	FB, F	
0.83	0.4	0.0	FB, F	2-CH_2OH, 3-C=O Furadan phenol

[a] FB = diazonium fluoroborate.
F = fluorescence.
CA = chromotropic acid reaction.
N = ninhydrin.

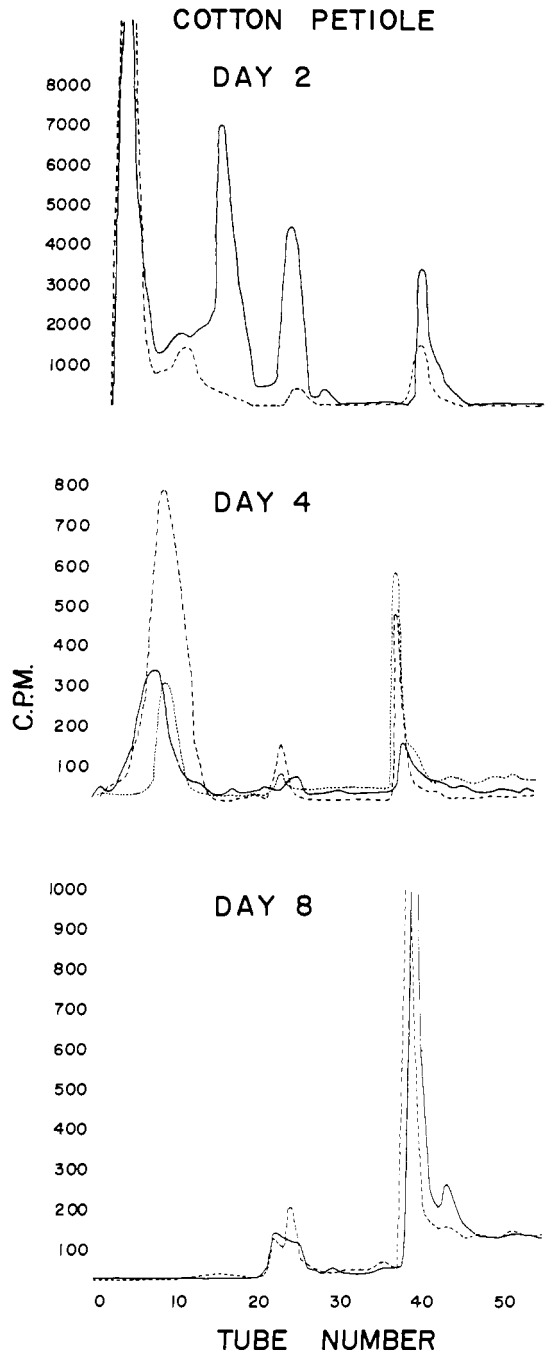


Figure 2. Eluted C^{14}- and H^3-labeled Furadan metabolites from column chromatography of extracts from isolated cotton leaves

——— *N*-$C^{14}H_3$
····· Ring C^{14}
- - - - - H^3

The production of the 3-OH Furadan is the first step in the plant oxidative metabolism, and this compound was always present in substantial amounts in the early stages of the isolated leaf experiments. The amount formed was dependent upon the metabolic activity of the leaf, and in one experiment, after 4 days 93% of the total eluted radioactivity from the column was the 3-OH Furadan, and this declined to 39% after 8 days. Generally, as shown in Figure 3, much lower relative amounts were present. Thus, the 3-OH Furadan is the key intermediate in the conversion of the parent Furadan into its further oxidation product the 3-C=O Furadan.

3-OH Furadan Phenol or 2,2-dimethyl-3,7-dihydroxy-2,3-dihydrobenzofuran (V) is the hydrolysis product of the 3-OH Furadan. This compound elutes in ether-hexane, and has an R_f 0.42 to 0.49 in TLC with EH. It was found only in trace amounts of TLC of some of the total leaf extracts.

3-C=O Furadan or 2,2-dimethyl-3-keto-2,3-dihydrobenzofuranyl-7 *N*-methylcarbamate (III) elutes in ether-hexane and is characterized by its intense blue-violet fluorescence which results from the resonance structures associated with the 3-benzofuranone ring. This compound was consistently found in the ether-hexane eluate after TLC in EH as a blue-violet F spot, R_f 0.35. Its identity has been confirmed by detection of its 2,4-dinitrophenylhydrazone as a brownish spot, by cochromatography with the synthetic model in several solvents, and by its infrared spectrum (Figure 1). The 3-C=O Furadan is hydrolytically unstable (Table III), and it does not accumulate to large quantities in the plant. Typical amounts found in the isolated leaf experiments ranged from 28.7% of the total radioactivity at 4 days to 10% at 8 days.

3-C=O Furadan Phenol or 2,3-dihydro-2,2-dimethyl-3-keto-7-hydroxybenzofuran (VI) is the hydrolysis product of the 3-ketocarbamate. This compound elutes from the column in ethyl acetate, and is strongly blue fluorescent. It can be distinguished from the 3-C=O carbamate by the distinct difference in fluorescent color (Table II) and by the R_f 0.50 to 0.59 by TLC with EH solvent. It gives an immediate red color with FB reagent without prior treatment with sodium hydroxide. This metabolite was consistently found in small amounts, and in one experiment comprised 1.1% of the total radioactivity from the column after 4 days (Figure 3). Its identity was unequivocally established by infrared and mass spectrometry of its glucoside as described below.

Conjugate Metabolites. Plants readily conjugate phenolic and other alcoholic substances, forming glucosides. As shown above, at least four such hydroxyl-containing substances have been identified as metabolic products of Furadan. Figure 2 shows that the amounts of polar metabolites eluting in methanol substantially increased with time of exposure of the radiolabeled carbamate in the cotton leaf, and after 8 days these became the major constituents of the leaf. These polar compounds have R_f 0.0 in EH solvent, and their radioactivity partitions about 0.1 in chloroform-water and 0.08 in benzene-water. These polar metabolites are found with both the ring-labeled and the *N*-$C^{14}H_3$-labeled Furadan (Figure 2) showing that at least one compound contains the carbamyl ester linkage. However, a large portion of the radioactivity at R_f 0.0 is distinguished by the intense blue-white fluorescence characteristic of the 3-ketobenzofuran (benzofuranone) nucleus.

These methanol eluting metabolites are resolved by TLC in BEW solvent (Figure 3) to give three major FB-positive spots with R_f values of approximately 0.25, 0.60, and 0.70. The spot R_f 0.60 has strong blue-white fluorescence and

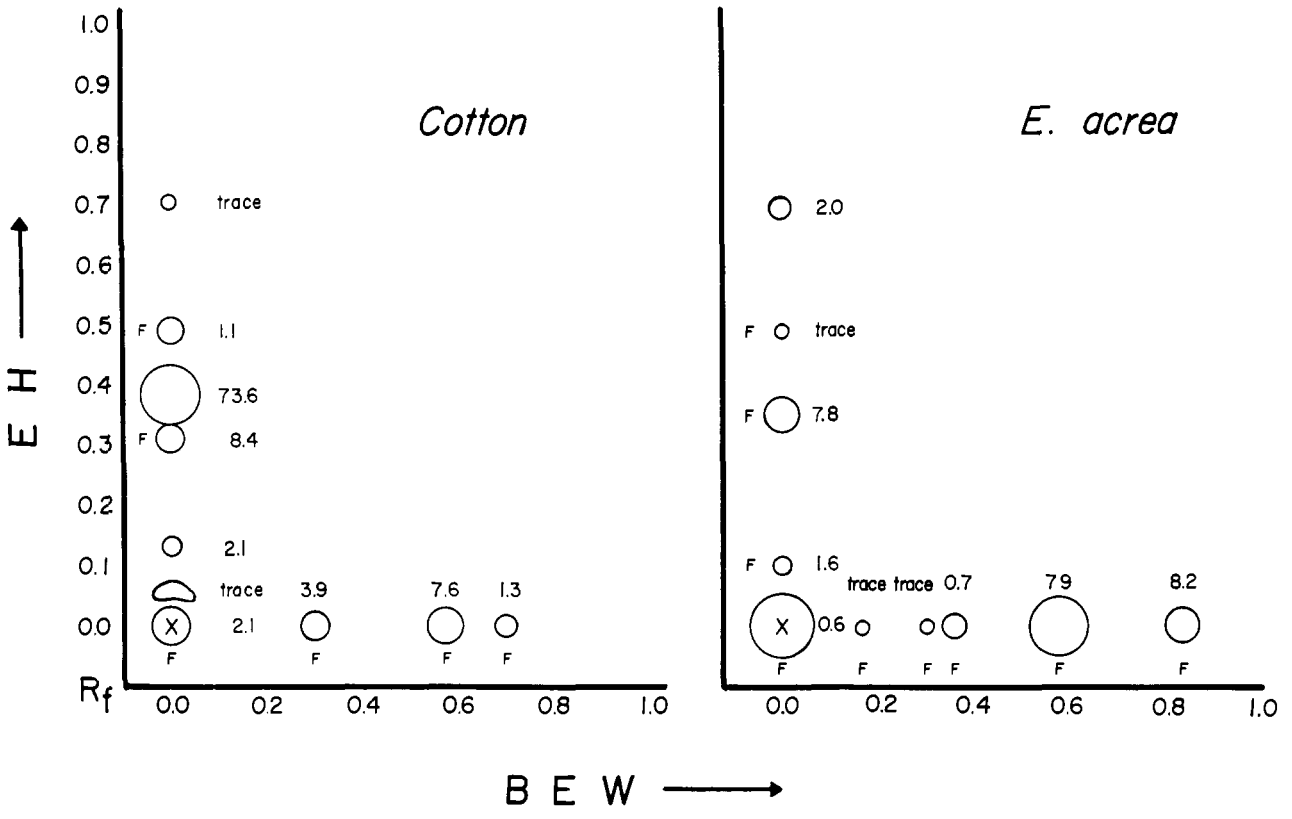


Figure 3. Replicas of two-dimensional thin-layer chromatograms showing C^{14}-ring labeled metabolites of Furadan from cotton petiole after 4 days and from salt marsh caterpillar (*E. acrea*) feces

appears to be the major polar metabolite. With the ring C^{14}-labeled Furadan in isolated cotton petioles, all three spots were radiolabeled. However, with *N*-$C^{14}H_3$-labeled Furadan, only the spot R_f 0.25 to 0.30 was radiolabeled.

The total R_f 0.0 materials from TLC in EH solvent from experiments with both ring and *N*-$C^{14}H_3$ labels were heated for 16 hours on the steam bath with 0.12 to 0.5*N* HCl, and the ether extracts were rechromatographed in EH solvent. The aglycones of the hydrolyzed conjugates were identified by comparison and cochromatography with the model compounds. The following conjugates were tentatively identified: 3-OH Furadan (X) R_f 0.25 to 0.30 BEW, R_f after hydrolysis 0.15 EH, yellow with FB, faint blue F, and the only conjugate radioactive with *N*-$C^{14}H_3$ label. This conjugate showed anticholinesterase activity by the blood plasma cresol red technique (Oonnithan and Casida, 1966), and gave a positive ninhydrin reaction. When the spot was extracted from the TLC plate, it gave an I_{50} for fly head ChE of $3.6 \times 10^{-6}M$ in the Warburg apparatus. All of these features indicate the presence of the intact *N*-methylcarbamate group.

The conjugate of the 3-OH Furadan phenol (VIII) R_f 0.70 BEW, after hydrolysis, gave R_f 0.52 in EH, and produced an immediate red color with FB. The conjugate of 3-C=O Furadan phenol (IX) R_f 0.60 in BEW, after hydrolysis gave R_f 0.61 in EH, and exhibited intense blue F. A small amount of the conjugate of Furadan phenol (VII) was also present, and the free phenol was identified after hydrolysis at R_f 0.80 in EH and immediate red color with FB. Possibly a diconjugate of 3-OH Furadan phenol might be formed, but its presence was not detected. Although Kuhr and Casida (1967) have shown that from 0.3 to 2.9% of the total radioactivity in bean plants injected with Banol, Baygon, and carbaryl was present as *N*-hydroxymethyl conjugates, such conjugates were not identified in the present study, perhaps because Furadan is less readily converted to *N*-CH_2OH derivatives than these other carbamates (Metcalf *et al.*, 1967), and because of the greatly increased hydrolytic instability of these *N*-CH_2OH carbamates (Table III).

The methanol eluting conjugates at R_f 0.0 in EH were also moistened with a few drops of β-glucosidase (emulsin) as a 10% solution in 0.0667*M* phosphate buffer, pH 6.85. After the spots had dried, they were chromatographed in EH solvent, and the 3-OH phenol R_f 0.39 and 3-C=O phenol *R*, 0.52, with strong blue F, were readily identified. The conjugate with the 3-OH Furadan was apparently considerably more stable than the phenolic conjugates, and only a small amount of the aglycone was liberated by the enzymatic treatment or by 1 to 2 hours' hydrolysis in HCl. The specificity of the β-glucosidase enzyme indicates that these plant conjugates are glucosides.

The identities of the 3-C=O Furadan phenol and its glucoside conjugate were unequivocally demonstrated by infrared and high resolution mass spectrometry. Milligram quantities of the conjugate were purified by TLC in EH and BEW systems, giving a very viscous liquid with a strong blue F. This was examined by infrared as a KBr pellet giving the spectrum shown in Figure 1. The absence of the NH plane deformation at 635 $cm.^{-1}$ and NH stretch-

ing at 3350 cm.$^{-1}$ demonstrates the hydrolytic separation of the *N*-methylcarbamoyl group. The presence of the 2,2-dimethyl-2,3-dihydro-3-ketobenzofuran moiety is established by the 1710 cm.$^{-1}$ (s) C=O; 1285 cm.$^{-1}$ (w) and 1232 cm.$^{-1}$ (m) the C—O—C furan ring; the 1379 cm.$^{-1}$ (s) and 1360 cm.$^{-1}$ (w) the *gem*-dimethyl groups of the furan ring; and the characteristic bonds for the 3-ketobenzofuran group 909 cm.$^{-1}$ (m), 835 cm.$^{-1}$ (w), 799 cm.$^{-1}$ (m), 748 cm.$^{-1}$ (m), 695 cm.$^{-1}$ (m), and 629 cm.$^{-1}$ (w). The glucose moiety is represented by overlapping groups of bonds associated with ring and —C—O stretching 1165 cm.$^{-1}$ (w), 1115 cm.$^{-1}$ (m), 1095 cm.$^{-1}$ (m), 1070 cm.$^{-1}$ (s), 1050 cm.$^{-1}$ (s), and 1029 cm.$^{-1}$ (m).

The presence of the 2,2-dimethyl-2,3-dihydro-7-hydroxybenzofuran-3-one moiety (P) was established by mass spectrometry to give the following molecular ions: *m/e* 178 (P^+), 179 $(P + 1)^+$, 177 $(P - 1)^+$, 163 $(P - 15)^+$ loss of a $—CH_3$, 161 $(P - 17)^+$ loss of a —OH, 150 $(P - 28)^+$ loss of C=O, and 120 $(P - 58)^+$ loss of two $—CH_3$ groups and C=O. The glucose moiety was established by *m/e* 339 $(M - 1)^+$, 145 $(P_G - 17)^+$ loss of —OH, 131 $(P_G - 31)^+$ loss of $—CH_2OH$, 113 $(P_G + 1) - 49)^+$ successive loss of water and CH_2OH, 85 loss of water from four-carbon fragment.

The conjugated metabolite was silated at room temperature in anhydrous pyridine with hexamethyldisilazane and trimethylchlorsilane. Mass spectrometry showed a molecular ion 628 mu and a spectrum characteristic of the polytrimethyl silylether. This agrees with the theoretical molecular weight of 628 for the completely silated 2,2-dimethyl-2,3-dihydrobenzofuran-3-one-7 glucoside.

Paper Electrophoresis was also used to characterize the conjugate metabolites of the various phenols, as Darby *et al.* (1966) have shown that in 0.05*N* NaOH, 1 naphthyl glucosiduronate and glucoside migrate to the cathode, and 1-naphthyl sulfate and phosphate to the anode. Whatman No. 1 paper, 0.16 mm. thick, was used for the electrophoresis with 0.05*N* NaOH buffer. At 400 volts and 100 ma. over 3.5 hours, the 3-keto-Furadan did not migrate, the 3-keto-Furadan-phenol migrated 12 cm. toward the anode, and the purified blue fluorescent water-soluble metabolite from *E. acrea* migrated 4 cm. toward the cathode. Similar experiments with the methanol-eluting, water-soluble cotton metabolite showed a similar blue fluorescent compound migrating toward the cathode. This, together with data presented above, shows that the 3-ketophenol is conjugated in both plants and in *E. acrea* as a glucoside.

METABOLISM OF 3-OH FURADAN IN COTTON LEAVES

Supplementary investigations were performed by allowing cotton leaf petioles to imbibe this primary Furadan metabolite, and separating the metabolic products by column and TLC as described above. *N*-C^{14}-CH_3-labeled 3-OH Furadan was obtained by ethyl acetate elution of the corresponding C^{14}-labeled Furadan following metabolism in cotton leaves. This was purified by TLC, and fortified with 1 mg. of the pure, unlabeled 3-OH Furadan. Three days after imbibation by the cotton petiole, column chromatography of the 70% ethanol extract showed 7.2% of the C^{14} eluting in ether-hexane, 2.8% in chloroform, 10.2% in ethyl acetate, and 28.7% in methanol. TLC of the leaf extract in EH showed nearly all the radioactivity at R_f 0.0, indicating the presence of conjugates, with traces of C^{14} and FB reactions at R_f 0.15, 3-OH Furadan. The area R_f 0.0 was refluxed for 16 hours in 0.5*N* HCl, and then extracted with ether. TLC of the ether extract with EH showed C^{14} only at R_f 0.15 which cochromatographed with the standard 3-OH Furadan in both EH and EB solvents. This experiment provides additional proof of the formation of a conjugate of the 3-OH Furadan.

Unlabeled metabolites of 3-OH Furadan were also detected by TLC in EH. A bright blue F compound had an R_f almost identical to 3-OH Furadan, but was separable in EB solvent. This compound also formed in small amounts in the Udenfriend oxidation of Furadan and is apparently 3-C=O, 6-OH Furadan. It did not give the chromotropic acid reaction, and thus was not an *N*-CH_2OH derivative. Also detected were 3-C=O Furadan and 3-C=O Furadan phenol, both free and conjugated.

METABOLISM IN INTACT COTTON PLANT

Several sets of experiments were performed with C^{14}-ring, *N*-$C^{14}H_3$, and H^3-labeled Furadan as a systemic insecticide in mature cotton plants about 1 meter high. The radiotracer was placed in a small capillary funnel cemented to a hole in the lower stem of the plant, and acetone-water added until the compound was absorbed. At intervals of 5, 14, and 30 days, the upper leaves were sampled, and homogenates, in 70% ethanol, chromatographed on a Florisil column, and eluted with the sequence of solvents previously described.

The over-all rate of metabolism in the intact plant was considerably more rapid than in the isolated leaves which had much higher relative concentrations of carbamate. In the *N*-$C^{14}H_3$ experiments, after 5 days the average concentration of C^{14} in the upper leaves was 0.83 p.p.m., and column chromatography showed none eluting in ether-hexane, about 12% in ethyl acetate, and 88% in methanol. After 14 days, the average concentration in the leaves was 1.3 p.p.m., none eluted in ether-hexane, 16% in ethyl acetate, and 84% in methanol. After 30 days, the average concentration in the leaves was 10 p.p.m., and 96% eluted in the methanol fraction. Similar results were found with C^{14}-ring labeled compound. Isolation by column and TLC showed the rapid oxidation to the 3-OH Furadan and subsequent oxidation through the 3-C=O Furadan followed by hydrolysis and conjugation of the phenols as the polar methanol metabolites.

When an intact cotton plant was treated with 5 mg. of *N*-$C^{14}H_3$ Furadan through a capillary funnel, the methanol-eluting C^{14} fraction (R_f 0.0 in EH) showed strongly radioactive spots at R_f 0.52 (violet F) and R_f 0.59 (blue F) in TLC with BEW. Both spots were red with FB, and neither cochromatographed with 3-C=O Furadan glucoside R_f 0.65. Upon hydrolysis of the concentrated C^{14} methanolic fraction with β-glucosidase, TLC in EH gave a blue F spot at R_f 0.15. This material was also found in the isolated cotton leaf experiments, and is not the 3-OH Furadan which has a very similar R_f, as the two compounds can be separated by TLC in EB. This aglycone is very similar in R_f and F to the product R_f 0.10 to 0.15 from the Uden-

friend oxidation (Table IV). The latter, by R_f and comparison with model compound 2,2-dihydro-3-keto-6-hydroxybenzofuranyl-7 *N*-methylcarbamate, is thought to be 3-C=O, 6-OH Furadan. The blue F minor plant metabolite is suspected, therefore, as being a conjugate of a ring-hydroxylated 3-C=O Furadan (XII).

METABOLISM IN ISOLATED CORN LEAVES

Experiments similar to those described for isolated cotton leaves were repeated with 10-day-old corn seedlings cut off above the roots, and placed in C^{14}-ring labeled Furadan solution. The rate of metabolism in corn was slower than that observed in cotton leaves under similar environmental conditions, and after 4 days (Figure 4) 45.0% of the total extracted radioactivity was found in the ether-hexane eluate, 8.3% in the chloroform eluate, 26.2% in the ethyl acetate eluate, and 20.5% in the methanol eluate. TLC of the ether-hexane eluate showed four fluoroborate positive spots: R_f 0.32, violet F, the 3-C=O Furadan; R_f 0.43, blue F, the 3-C=O Furadan phenol; R_f 0.38, Furadan (85%); and R_f 0.72, the Furadan phenol. TLC of the ethyl acetate eluate showed a strong FB reaction at R_f 0.1 (95%). This spot was identified as 3-OH Furadan as it was oxidized by CrO_3 to give the violet F 3-C=O Furadan; and a trace spot at R_f 0.5, probably the 3-C=O Furadan phenol. TLC of the methanol eluate in EH gave a blue F spot at R_f 0.0. This was resolved by TLC in BEW at R_f 0.0, 0.27, 0.43, and 0.66. These appear to be the same conjugate metabolites discussed under cotton metabolites.

Corn grown from seeds treated topically with 80 μg. of H^3-labeled Furadan was harvested after 32 days, when the leaves contained about 34% of the applied dosage and the roots about 2.6%. TLC showed the presence of the 3-OH Furadan and 3-C=O Furadan phenol in the leaves together with the phenolic conjugates. The roots contained a large proportion of Furadan phenol and smaller amounts of 3-OH Furadan and 3-C=O Furadan, suggesting that oxidation is more rapid under photosynthetic conditions.

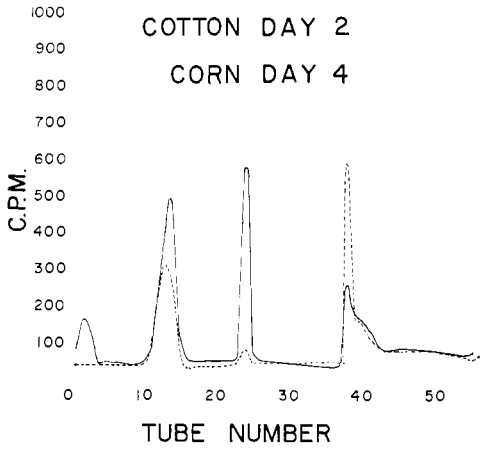


Figure 4. Comparison of elution chromatograms of C^{14}-ring labeled Furadan metabolites

------- In cotton
——— In corn leaves

METABOLISM IN INSECTS

The metabolism of carbamate insecticides in insects is remarkably similar to that in plants and in higher animals (Dorough and Casida, 1964; Metcalf *et al.*, 1966). In the housefly, *N*-$C^{14}H_3$ labeled Furadan was only very slowly metabolized to $C^{14}O_2$, an average of about 0.7% of the absorbed radioactivity following topical application being converted to $C^{14}O_2$ in 24 hours, as compared with approximately 2% for the closely related *o*-isopropoxyphenyl *N*-methylcarbamate (Baygon) (Metcalf *et al.*, 1967). Therefore, there is only trace metabolism in the housefly to the *N*-CH_2—OH derivatives of Furadan and its oxidation products. C^{14}-ring labeled Furadan, under identical circumstances, produced only a trace of $C^{14}O_2$, <0.1% of the absorbed dosage in 24 hours, indicating that the aromatic ring is not appreciably attacked in the detoxication process.

Experiments with radiolabeled Furadan ingested by larvae of the salt marsh caterpillar, *Estigmene acrea*, have given essentially the same metabolic pattern as in cotton leaves. The compound was placed on cotton leaves as a residue or in them by translocation through the petiole. The feces of the larvae were extracted in acetone or ethanol and subjected to chromatographic analysis. In a typical experiment, 1 mg. of C^{14}-ring labeled Furadan was taken up in a cotton petiole, and fed to four starved fifth instar larvae over a 7-hour period. Two-dimensional TLC, using EH and BEW solvents, gave the pattern shown in Figure 3. The results were qualitatively very similar to those found with the cotton leaf (Figure 3). However, the degradation was much more rapid, and none of the parent carbamate was recovered. There were 7.8% 3-C=O Furadan and 2.0% of what appeared to be Furadan phenol (R_f 0.7). Approximately 88% of the total C^{14} was present as the highly polar conjugates R_f 0.0 in EH solvent. The major conjugate, 79% of the total C^{14}, was the blue F metabolite R_f 0.6 in BEW solvent. This was isolated in large quantities by feeding the larvae 50 mg. of 3-C=O Furadan or 10 mg. of 3-C=O Furadan phenol, both of which were converted almost quantitatively to the 3-C=O Furadan phenyl glucoside which was identified by its hydrolysis by β-glucosidase to the 3-C=O Furadan phenol, and by its cochromatography with, and identical infrared spectrum to 2,2-dimethyl-2,3-dihydrobenzofuran-3-one-7-yl glucoside from cotton leaves.

Repetition of this experiment, using 1 mg. of C^{14}-ring labeled Furadan applied from acetone to the surface of a cotton leaf and fed to five starved *E. acrea* larvae, resulted in an identical pattern of metabolism. After 16 hours, 93% of the C^{14} in the feces was present as conjugates R_f 0.0 in EH solvent, and the remainder as traces of 3-OH Furadan and Furadan. More than 90% of the conjugates was blue F 3-C=O Furadan phenyl glucoside, R_f 0.61 BEW solvent.

When 50 mg. of 3-OH Furadan were fed to 20 *E. acrea* larvae, the feces contained polar conjugates (R_f 0.0 in EH solvent), and considerable amounts of 3-OH Furadan phenol (R_f 0.45) together with lesser amounts of 3-OH Furadan (R_f 0.15), 3-C=O Furadan (R_f 0.32), and 3-C=O Furadan phenol (R_f 0.57). The conjugates when hydrolyzed with β-glucosidase liberated the 3-OH Furadan phenol and the 3-C=O Furadan phenol. The con-

jugate of 3-OH Furadan was not positively identified, suggesting that this compound is so readily oxidized and hydrolyzed that little of the conjugated 3-OH Furadan is formed.

Similar experiments were 50 mg. of 3-C=O Furadan were fed to *E. acrea* produced feces containing the 3-C=O Furadan, the 3-C=O Furadan phenol, and a large quantity of conjugates (R_f 0.0 in EH solvent) which were resolved in BEW solvent to the blue F 3-C=O Furadan phenyl glucoside (R_f 0.60), and a violet F spot (R_f 0.20) whose infrared spectrum did not show the glucose moiety.

To determine the extent of metabolism by *N*-dealkylation, 0.5 mg. of *N*-$C^{14}H_3$ Furadan was fed to five last instar *E. acrea* larvae. During a 24-hour period, 2.45% of the total absorbed dosage was recovered as $C^{14}O_2$, using the method of Metcalf *et al.* (1967). Thus, this metabolic pathway is a minor one in this insect. In a further experiment, 2 mg. of *N*-CH_2OH Furadan was applied to a cotton leaf disk and fed to five *E. acrea* larvae. The acetone extract of the feces was subjected to TLC as previously described. In EH solvent, no FB positive compounds moved from the blue F origin. Hydrolysis of this spot in 0.5*N* HCl, followed by extraction with ether, and TLC in EH solvent gave a FB-positive spot at R_f 0.85 which was apparently Furadan phenol. The material remaining at the origin gave a strong violet reaction with CA, and there was also a faint violet CA reaction at R_f 0.30 which was pink with FB after sodium hydroxide. These facts suggest ready hydrolysis of *N*-CH_2OH Furadan to Furadan phenol and subsequent conjugation. This is to be expected by the relative hydrolytic instability of *N*-CH_2OH Furadan (Table III). Another metabolic pathway is by further oxidation to the 3-C=O Furadan. However, no trace of 3-C=O, *N*-CH_2OH Furadan R_f 0.12 was found.

METABOLISM IN THE WHITE MOUSE

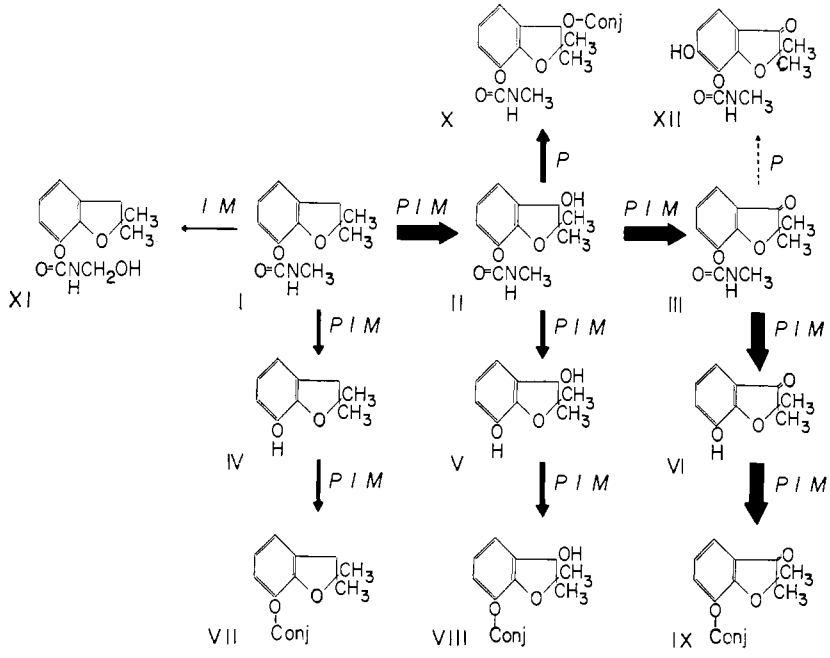
Male Swiss white mice deprived of food and water for 1 hour were treated orally with 0.1% w./v. radiolabeled Furadan in propylene glycol using a microsyringe and blunt needle. Urine was collected over a 24-hour period, and in some experiments exhaled $C^{14}O_2$ was trapped. The high toxicity of the compound, oral LD_{50} about 2 mg. per kg., made it difficult to obtain large amounts of radioactive metabolites from the C^{14}-radiotracer, and most satisfactory results were obtained with the H^3-radiotracer. The collected urine was counted for total radioactivity, extracted with an equal volume of diethyl ether, and the ether extract concentrated and examined by TLC. The aqueous fraction was hydrolyzed for 16 hours with 0.12*N* HCl on the steam bath and again extracted with ether, and the ether extract examined by TLC.

The two mice treated orally with 2 mg. per kg. of H^3-labeled Furadan eliminated 37% and 67% of the administered radioactivity in the urine in 24 hours. In another experiment with *N*-$C^{14}H_3$-labeled Furadan, 50% of the radioactivity was eliminated in 24 hours. In this experiment, about 1.6% of the total C^{14} was recovered as $C^{14}O_2$,

Table VI. Ether-Extractable H^3-Labeled Furadan Metabolites in Mouse Urine

R_f–EH 3 to 1	C.P.M.	Reactions[a]	Probable Identity
0.0	143	Blue F	Conjugate of 3-C=O Furadan
0.17	2496	FB	3-OH Furadan
0.30	645	Light violet F	
0.45	191	Violet F	3-C=O Furadan
0.63	74	Blue F	3-C=O Furadan phenol
0.82	978	FB	Furadan phenol

[a] See Table V for symbol designation.



Figures 5. Pathways of Furadan metabolism

P. In plants. I. In insects. M. In mammals. Broad arrows represent major pathways

suggesting a low rate of metabolism through the N-CH_2OH derivatives.

The ether extracts of mouse urine showed, after TLC in EH solvent, the distribution of radioactivity indicated in Table VI. The major component, R_f 0.17, was the 3-OH Furadan, and there was also a small amount of 3-C=O Furadan, R_f 0.45, and an appreciable amount of Furadan phenol, R_f 0.82.

The aqueous portion of the extracted urine contained only conjugates, R_f 0.0 in EH solvent. The major blue F component migrated to R_f 0.51 in BEW solvent, and was the conjugate of the 3-C=O Furadan phenol. After acid hydrolysis of the aqueous fraction, H^3-labeled spots were found in TLC with EH solvent at R_f 0.16, probably 3-OH Furadan, R_f 0.57, the 3-C=O Furadan phenol; R_f 0.72, Furadan phenol; and two unidentified compounds R_f 0.30 and 0.44. Thus, the metabolism in the mouse appeared to follow the same general oxidative pathways as in plant and insect (Figure 5). However, considerably more of the compound appeared to be eliminated by the mouse as the water-soluble 3-OH carbamate.

PHOTODECOMPOSITION OF FURADAN

Residues of Furadan on plant surfaces are subjected to photochemical action which affects the insecticidal action of the carbamate and its residual behavior. Preliminary investigations were made of the effects of fluorescent light and sunlight on residues of crystalline Furadan in petri dishes. At intervals, samples of residues were removed, and subjected to TLC as described above. Two days of outdoor sunlight produced a fluoroborate-positive spot at R_f 0.14 EH which appears to be the 3-OH carbamate. This same material also appeared in plates exposed to fluorescent light at 70°F. in an environmental chamber after one week. After two and one-half weeks, three other spots R_f 0.54, 0.71 and 0.74 appeared in the samples irradiated in sunlight. The compound R_f 0.71 did not inhibit cholinesterase and was probably the Furadan phenol. No trace of the 3-C=O carbamate appeared after exposure of Furadan in thin-layer for 2 weeks.

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