Metabolism of Methazole in Wheat and Onions

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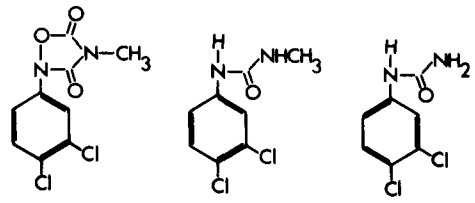
Methazole, 2-(3,4-dichlorophenyl)-4-methyl-1,2,4-oxadiazolidine-3,5-dione, is a herbicide under development by the Velsicol Chemical Corp. Because of its efficacy in controlling weeds in cotton, the metabolism of the compound in cotton has been studied (JONES and FOY, 1972; DOROUGH et al., 1973). Field evaluations also have demonstrated that methazole is an effective herbicide for use in the production of wheat and onions, and commercial development for this purpose is anticipated (WHITACRE, 1973). This being the case, it is necessary that the metabolic fate of methazole in wheat and onions be defined so that the nature, levels and toxicological significance of residues on these crops can be determined.

MATERIALS AND METHODS

Chemicals. Radioactive preparations of methazole used in these studies and designations of each used in the text were methazole-phenyl-^{14}C (sp. act. 13.5 mCi/mmol), methazole-3-^{14}C (sp. act. 7.7 mCi/mmol) and methazole-5-^{14}C (15.7 mCi/mmol). In addition, the methylurea derivative of methazole [3-(3,4-dichlorophenyl)-1-methyl-urea (carbonyl-^{14}C] was included and is designated as methazole-methylurea-^{14}C; its sp. act. was 9.6 mCi/mmol. The urea analog of methazole [3-(3,4-dichlorophenyl) urea] was a common metabolic product and is referred to as methazole-urea in the text. Non-radioactive authentic samples of methazole, methazole-methylurea and methazole-urea were available for comparison with metabolites produced in the wheat and onions. Structures of these compounds are shown below.



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Treatment and Extraction of Plants. Wheat plants (Arthur variety) 6 to 8 in. in height and Burmuda onions 8 to 10 in. tall, with a bulb of approximately 1/2 in. in diameter, were placed individually in 10 ml of water containing 1 x 10^6 dpm of the radioactive methazole or methazole-methylurea-^{14}C for 24 hrs. The plants were then taken from the treatment solution, washed with water, and transferred to distilled water until removed for analysis. Four plants were used for each designated time interval for all treatments.

Individual whole plants were chopped into small pieces and homogenized thoroughly in 20 ml of methanol using a Brinkman Polytron homogenizer. After filtering, the plant solids were again extracted with methanol, the extracts combined and then concentrated to a volume suitable for application to a tlc plate. The plant solids were added to 20 ml of 1N HCl, heated at 90°C for 1 hr., cooled, and the solution neutralized with sodium hydroxide and filtered. The filtrate was extracted 3 times with 10 ml-volumes of ethyl acetate and the extracts analyzed by tlc.

Thin Layer Chromatography and Radioassay. Silica gel F_{254} precoated chromatoplates (Merck) were developed in a 7:2:1 mixture of petroleum ether-chloroform-ethanol. Aluminum oxide F_{254} chromatoplates (Merck) were developed in a 9:1 mixture of ethyl acetate-isopropyl alcohol. The chromatographic behavior of methazole and its metabolites in these solvent systems were described in an earlier report (DOROUGH et al., 1973). Radioautography was used to locate the radioactive compounds on the gel while the non-radioactive standards were visualized under ultra violet light.

A Packard Tri-Carb Model 3380-544 scintillation counter was used for all radioassays. Plant solids were combusted in a Beckman Biological Materials Oxidizer and the ^{14}-carbon dioxide trapped in a 2 to 1 solution of methylcellosolve and ethanolamine for counting.

RESULTS AND DISCUSSION

Methanol extracts of wheat and onion plants treated with methazole-phenyl-^{14}C contained up to 7 radioactive components which were resolved by tlc in the 7:2:1 petroleum ether-chloroform-ethanol solvent system. The R_f values for the compounds, designated as metabolites I through VII, were I = 0.0, II = 0.07, III = 0.17, IV = 0.33, V = 0.55, VI = 0.68 and VII = 0.82.

Metabolites I and II yielded a mixture of methazole-methylurea and methazole-urea, plus trace amounts of several other products, when treated with 1N HCl for 1 hr at 90°C. This same situation was noted with metabolites from cotton (DOROUGH, et al., 1973) having R_f values identical to metabolites I and II from wheat and onions. As suggested in the previous study, the metabolites appear to be composed largely of salts of the methylurea and urea derivatives of methazole.

Metabolites III, IV and VII were identified as methazole-urea, methazole-methylurea and methazole, respectively, based on cochromatography of the radioactive metabolites with the appropriate

authentic standard in several tlc solvent systems. In addition, isotopic dilation analysis (repeated recrystallization of the standard from an ethyl acetate-hexane solution containing the radioactive metabolite) showed that constant specific activities were obtained when the standard was recrystallized in the presence of the metabolite having an identical R_f value on tlc. Specific activities declined with each crystallization when the standard was mixed with radioactive metabolites having different R_f values.

Because metabolites V and VI were so low in concentration their identities were not ascertained. They were, however, chromatographically similar to 6,7-dichloro-1-methyl-2-benzimidazolinone and its 5,6-dichloro analog. These materials were identified as photoproducts of methazole by IVIE et al. (1973).

Acid treatment of the plant solids after extraction with methanol produced metabolites I, III and IV. No attempts were made to identify metabolite I, that radioactivity remaining at the tlc origin, but metabolites III and IV were identified as methazole-urea and methazole-methylurea using the techniques described above.

The concentrations of methazole-phenyl-^{14}C and its metabolites in wheat and onion seedlings at 1, 6 and 12 days after removal from the treatment solution are shown in Table 1. Qualitatively, the metabolic pathways of methazole in these plant species were the same, and were the same as reported for cotton and beans (DOROUGH et al., 1973). Quantitatively, however, there were some major differences. For example, methazole was completely metabolized in wheat by 1 day after treatment, while 32% of the radiocarbon absorbed by the onion plants remained as the parent compound. In wheat, the predominant metabolites were the methylurea and urea derivative of methazole. Together they accounted for 50 to 60% of the dose at each sampling interval and were present at similar concentrations. On the other hand, methazole-urea was not detected at all in the 1-day onion plants, and at very low levels in the 6- and 12-day samples. Methazole-methylurea was the major metabolite in the onions at each sampling time where it constituted at least 40% of the applied dose.

Generally, the unknown metabolites in the methanol extracts were of similar concentrations in both wheat and onions (Table 1). However, metabolite II was at consistantly greater concentration in the onions than in the wheat and, with the exception of the 12-day sample, metabolite I was greater in the wheat. Since acid treatment of metabolite I gave predominately methazole-urea and low levels of methazole-methylurea, with the reverse being true of metabolite II, the concentrations of metabolites I and II are probably dependent upon the levels of the free methylurea and urea formed in the plants. This being the case, the higher concentrations of metabolite I in the wheat and of metabolite II in onions would be expected.

Acid treatment of the wheat solids released an additional 5-6% of the applied dose in the 1, 6 and 12-day plants (Table 1). About 50% of the released radiocarbon was as methazole-urea and about 30% as methazole-methylurea. The remainder of the radiocarbon did not

TABLE 1.

Fate of methazole-phenyl-^{14}C in wheat and onion seedlings[a].

Metabolite	% of applied dose/days after treatment					
	1		6		12	
	Wheat	Onions	Wheat	Onions	Wheat	Onions
Methanol Extractables						
I Unknown	5.7	2.5	4.5	1.3	4.1	4.2
II Unknown	2.8	9.0	1.7	2.8	2.1	6.7
III Methazole-urea	32.1	0	22.8	1.7	21.1	5.9
IV Methazole-methylurea	27.2	40.0	35.7	59.4	30.0	44.0
V Unknown	1.7	.9	2.1	1.8	2.9	1.7
VI Unknown	.8	.4	.6	.6	1.0	1.2
VII Methazole	0	32.3	0	6.8	0	1.1
Total	70.3	85.1	67.4	74.4	61.2	64.8
Acid Extractables						
I Unknown	1.2	2.5	.7	1.7	1.4	2.9
III Methazole-urea	2.7	.2	2.6	1.1	2.3	2.1
IV Methazole-methylurea	2.1	3.8	1.7	5.7	1.2	4.0
Total	6.0	6.5	5.0	8.5	4.9	9.0
Unextracted	14.2	6.2	12.7	13.0	14.9	22.5
Total recovery	90.5	97.8	85.1	95.9	81.0	96.3

[a] Wheat and onion seedlings placed in water solution of methazole-phenyl-^{14}C for 24 hrs, then transferred to fresh water. Each plant contained about 40% (4.0×10^5 dpm) of the total radiocarbon in the treatment solution.

move from the tlc origin. With onions, the acid extractables increased from 6.5% of the dose in the 1-day samples to 9% in the 12-day samples. The increase was attributable primarily to radiocarbon released as methazole-urea.

^{14}C-Residues which could not be extracted from the 1-day wheat plants, 14.2% of the dose, was approximately twice that in the onions. Residues of this nature remained at nearly the same level in the wheat throughout the experiment. In onions, they increase with time and accounted for 22.5% of the dose in the 12-day plants.

Total recovery of the radioactivity absorbed by the plants ranged from 81 to 90% in the wheat and from 96 to 98% in the onions. The lower recovery in the wheat, which declined with time, was apparently associated with the methanol extractables (Table 1). Because methazole-urea was the only product which occurred at high levels in the wheat but not in the onions, the partial loss of this material during sample preparation was suspected.

Treatment of wheat seedlings with methazole-3-^{14}C (Table 2) gave the same series of metabolites, and in similar concentrations, as treatment with methazole-phenyl-^{14}C. Methazole-5-^{14}C treatment of the wheat resulted in very low levels of radioactive metabolites in the plants. These experiments demonstrated that methazole was rapidly metabolized by the wheat to form methylurea and ^{14}-carbon dioxide as was reported for cotton and beans (DOROUGH et al., 1973). That almost identical results were obtained with the phenyl-^{14}C and 3^{14}-C methazole showed that little, if any, metabolism occurred which resulted in the cleavage of the No. 3 carbon from the phenolic moiety of the molecule. Therefore, it would be expected that the fate of methazole-methylurea in wheat would be essentially the same as methazole, per se. This was demonstrated to be the case in both wheat and onions treated with methazole-methylurea-^{14}C (Table 3). These studies also confirmed that wheat was much more effective in metabolizing methazole-methylurea to the urea derivative than was the onion.

Methanol extracts of wheat and onions were analyzed by tlc using the aluminum oxide plates to determine the presence of methoxymethylurea [3-(3,4-dichlorophenyl)-1-methoxy methylurea]. It was shown earlier (DOROUGH et al., 1973) that this product in a methanol extract was indicative of the presence of N-hydroxymethylurea [3-(3,4-dichlorophenyl)-3-hydroxy-1-methylurea] in the plants. The latter metabolite is unstable but, in the presence of methanol, is converted to methoxymethylurea which is stable. Methoxymethylurea has the same R_f as methazole-methylurea in the silica gel tlc system but the compounds are resolved on the aluminum oxide chromatoplates.

Methoxymethylurea-^{14}C was detected in extracts of both wheat and onions. Quantitatively, the results were quite variable with the metabolite accounting for 2 to 12% of the applied dose in both plants. Although not firmly established, it appeared that methoxymethylurea-^{14}C was at higher concentrations in the 6-day samples of both species, and was somewhat higher in onions than in wheat.

TABLE 2.

Fate of methazole-3-^{14}C and methazole-5-^{14}C in wheat seedlings[a].

	% of applied dose/days after treatment					
	1		6		12	
Metabolite	3-^{14}C	5-^{14}C	3-^{14}C	5-^{14}C	3-^{14}C	5-^{14}C
Methanol Extractables						
I Unknown	4.1	0.3	3.1	0.2	3.3	0.2
II Unknown	2.6	0	3.5	0	4.6	0
III Methazole-urea	36.0	0	30.3	0	22.7	0
IV Methazole-methylurea	29.7	0	31.1	0	33.8	0
V Unknown	.9	0	1.1	0	1.4	0
VI Unknown	.3	0	.5	0	.7	0
VII Methazole	0	1.5	0	1.3	0	0
Total	73.6	1.8	69.6	1.5	66.5	.2
Acid Extractables						
I Unknown	.5	.2	.3	0	1.4	.1
III Methazole-urea	2.4	0	1.6	0	2.5	0
IV Methazole-methylurea	1.5	0	1.0	0	2.1	0
Total	4.4	.2	2.9	0	10.6	.1
Unextracted	12.3	1.3	10.0	.9	15.7	1.2
Total recovery	90.3	3.3	82.5	2.4	92.8	1.5

[a] See footnote Table 1 for method of treatment. Uptake of methazole-3-^{14}C and -5-^{14}C by the seedlings were the same as for methazole-phenyl-^{14}C.

TABLE 3.

Fate of methazole-methylurea-^{14}C in wheat and onion seedlings[a].

	% of applied dose/days after treatment					
	1		6		12	
Metabolite	Wheat	Onions	Wheat	Onions	Wheat	Onions
Methanol Extractables						
I Unknown	4.6	0.9	1.6	2.4	2.3	2.5
II Unknown	3.5	3.1	3.1	2.2	1.8	2.7
III Methazole-urea	35.4	0	40.6	4.2	49.5	3.7
IV Methazole-methylurea	42.4	88.6	30.6	78.4	28.3	64.6
Total	85.9	92.6	75.9	87.2	81.9	73.5
Acid Extractables						
I Unknown	.2	.1	.5	.3	.2	.3
III Methazole-urea	2.3	0	2.1	.1	4.0	.1
IV Methazole-methylurea	2.1	3.7	1.0	3.2	1.5	4.7
Total	4.6	3.8	3.6	3.6	5.7	5.1
Unextracted	7.9	4.5	9.7	7.4	5.9	9.1
Total recovery	98.4	100.9	89.2	98.2	93.5	92.4

[a] Wheat and onion seedlings placed in water solution of methazole-methylurea-^{14}C for 24 hr and transferred to fresh water for specified time after treatment. Each plant contained approximately 25% (2.5×10^5 dpm) of the total radiocarbon originally added to the water solution at the time they were placed in fresh water.

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