J Plant Growth Regul (1982) 1:305–316

Journal of
Plant
Growth
Regulation

Comparative Effects of Methazole and Two of Its Degradation Products in Plants on the Metabolism of Enzymatically Isolated Leaf Cells of Velvetleaf (*Abutilon theophrasti* Medic)*

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Received October 14, 1982; accepted January 10, 1983

Abstract. Effects of methazole [2-(3,4-dichlorophenyl)-4-methyl-1,2,4-oxadiazolidine-3,5-dione] and of its plant degradation products, DCPMU [3-(3,4-dichlorophenyl)-1-methylurea] and DCPU [3-(3,4-dichlorophenyl)urea], on photosynthesis, protein synthesis, ribonucleic acid (RNA) synthesis, and lipid synthesis of enzymatically isolated leaf cells of velvetleaf (*Abutilon theophrasti* Medic) were compared. Photosynthesis and protein, RNA, and lipid synthesis were assayed by the incorporation of $NaH^{14}CO_3$, [^{14}C]-leucine, [^{14}C]-uracil, and [^{14}C]-acetate, respectively, into the isolated cells. Time-course and concentration studies included incubation times of 30, 60, and 120 min and concentrations of 0.1, 1, 10, and 100 μM of all three chemicals. DCPMU was a more potent inhibitor of the four metabolic processes examined than either the parent herbicide methazole or DCPU. The sensitivity of the four metabolic processes to DCPMU decreased in the order: photosynthesis > lipid > RNA > protein synthesis. Inhibition of all metabolic processes by methazole was time-dependent, increasing in magnitude with concomitant increases in incubation time. It is probable that the observed effects of methazole were caused by DCPMU, formed through metabolism of methazole by the iso-

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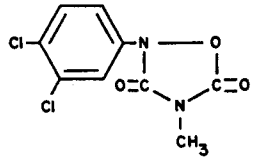
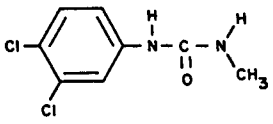
0171-7595/82/0001-0305 $02.40

lated leaf cells of velvetleaf rather than by methazole itself. DCPU was less active than the parent herbicide methazole and DCPMU and seems to be a terminal metabolite of methazole with limited phytotoxicity.

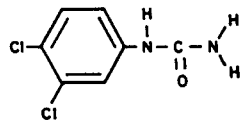
Methazole [2-(3,4-dichlorophenyl)-4-methyl-1,2,4-oxadiazolidine-3,5-dione] is a herbicide that has excellent potential for selective control of a wide spectrum of broadleaved weeds in several important crops including cotton (*Gossypium hirsutum* L.), alfalfa (*Medicago sativa* L.), and onion (*Allium cepa* L.) (Brockman 1975, Keeley et al. 1972, 1973, Keeley and Thullen 1974, Verity et al. 1981a, 1981b, 1981c, Whitacre and Whitehead 1976). A number of studies examining the patterns of methazole absorption, translocation, and metabolism in crop and weed plants, as well as its fate in the environment, are available (Brockman 1975, Brockman and Duke 1977, Butts and Foy 1974, Dorough 1974, Dorough et al. 1973, Ivie et al. 1973, Jones and Foy 1972, Keeley and Thullen 1979, Suzuki and Casida 1981, Verity et al. 1981a, 1981b, 1981c). These studies revealed that methazole is readily absorbed by roots and translocated through the xylem to the leaves, where it expresses its phytotoxic symptoms. Metabolism of methazole by plants yields two principal metabolites, identified as DCPMU [3-(3,4-dichlorophenyl)-1-methylurea] and DCPU [3-(3,4-dichlorophenyl)-urea], as well as some identified and unidentified polar metabolites. The chemical structures of methazole, DCPMU, and DCPU are shown in Fig. 1. DCPMU is a key metabolite in the metabolism of methazole by



METHAZOLE



DCPMU



DCPU

Fig. 1. Chemical structures of the herbicide methazole [2-(3,4-dichlorophenyl)-4-methyl-1,2,4-oxadiazolidine-3,5-dione] and its two metabolites, DCPMU [3-(3,4-dichlorophenyl)-1-methylurea] and DCPU [3-(3,4-dichlorophenyl)-urea].

plants, and its formation is believed to be the result of a NADPH-dependent reductive cleavage of the oxadiazolidinedione ring, probably at the N-O bond, decarboxylation, and reduction of N-OH-DCPMU, an intermediate metabolite, to DCPMU (Suzuki and Casida 1981). Recently, in metabolic studies of methazole with spinach (*Spinacea oleracea* L.) tissues, Suzuki and Casida (1981) identified the polar metabolites of methazole as the *N*-glucoside of the intermediate metabolite *N*-hydroxy-DCPMU.

Studies on the mode of action of methazole are limited. The symptoms of methazole phytotoxicity appear to include stunting and chlorosis followed by necrosis proceeding basipetally from the tips to the bases of leaves (Brockman and Duke 1977). These symptoms appear to resemble very closely those caused by herbicides that act as photosynthesis inhibitors. Preliminary studies by Brockman (1975) and more recently by Suzuki and Casida (1980, 1981) revealed that DCPMU was a much stronger inhibitor of the Hill reaction in isolated chloroplasts than was the parent herbicide methazole. Thus, the reductive cleavage of methazole to DCPMU appears to be a bioactivation pathway, in contrast to the oxidative *N*-demethylation of DCPMU to DCPU and the conjugation of *N*-OH-DCPMU with glucose, which are detoxification reactions. Differential rates of occurrence of these bioactivation or detoxification reactions that are involved in the plant metabolism of methazole have been suggested as the basis for the observed selectivity of this herbicide between tolerant and susceptible plants (Brockman 1975, Butts and Foy 1974, Keeley and Thullen 1979, Suzuki and Casida 1981, Verity et al. 1981c). However, apart from their effects on photosynthesis as measured by the Hill reaction of isolated chloroplasts, the effects of methazole, DCPMU, and DCPU on other plant physiological processes such as protein, ribonucleic acid (RNA), and lipid synthesis have not been examined. Many herbicides that are known to act as photosynthetic inhibitors are also capable of interfering with other physiological processes of plants (Ashton et al. 1977, Ashton and Crafts 1981). Therefore, additional studies on the mode of action of methazole and of its two metabolites are well justified. Recently, enzymatically isolated leaf cells of selected plant species have been used successfully in screening studies on the possible mode of action of several herbicides (Porter and Bartels 1977, Ashton et al. 1977, Hatzios and Penner 1980, Hatzios 1982). Velvetleaf (*Abutilon theophrasti* Medic) represents a plant species whose cells can be easily isolated enzymatically in an active state.

Consequently, the objectives of the studies reported herein were to determine and compare the effects of methazole, DCPMU, and DCPU on photosynthesis and protein, RNA, and lipid synthesis of enzymatically isolated leaf cells of velvetleaf during time-courses and with various concentrations of the compounds.

Materials and Methods

Plant Material

Seeds of velvetleaf (*Abutilon theophrasti* Medic) collected from a field located in Blacksburg, Virginia (USA), were mechanically scarified and planted in greenhouse soil mixture, one seed/473

ml plastic cup. The soil mixture was a 2:2:1 mixture of potting medium (Weblite Corporation, Blue Ridge, Virginia), vermiculite, and sphagnum peat moss containing also a controlled-release fertilizer (14-14-14) and limestone. The emerged seedlings were grown in a growth chamber with a photoperiod of 16 h (50–60 W m^{-2} supplied by fluorescent and incandescent lights) at 30°C and 8 h dark period at 20°C for 3 weeks. The photoperiod of the chamber was then changed to 6 hours. A short-day treatment has been reported to increase the photosynthetic rates of isolated leaf cells of cotton (Rehfeld and Jensen 1973) and of soybean (Servaites and Ogren 1977), since it reduces the amount of starch in the chloroplasts. After 1 week of short-day treatment, mature leaves of velvetleaf were used for isolation of mesophyll cells. On the day of cell isolation, mature leaves were detached from the plant after they had been illuminated for at least 1 hour.

Cell Isolation

Two to three grams of leaf tissue, cut in 1 mm × 1 mm strips with a razor served as the source for the enzymatic isolation of mesophyll cells through a 3-step procedure. The procedure included infiltration of the leaf tissue with the maceroenzyme (Macerase®, Calbiochem-Behring Corp., La Jolla, California) under vacuum, maceration of the infiltrated tissue through slow magnetic stirring, and repeated washings of the released cells through centrifugation. A detailed description of these procedures has been given elsewhere (Hatzios 1982). The released cells were made up to the desired volume with incubation medium. This medium contained 0.5 M sorbitol, 5 mM KNO_3, 2 mM $Mg(NO_3)_2$, and 1 mM $CaCl_2$ and was buffered with 50 mM HEPES, pH 7.8, for photosynthesis or with 50 mM MES, pH 5.8, for protein, RNA, and lipid synthesis assays. For the chlorophyll determination, 1 ml of the cell suspension was added to 4 ml of 80% aqueous acetone and mixed thoroughly. The supernatant fluid was then assayed spectrophotometrically for its chlorophyll content according to the method of Arnon (1949). The chlorophyll content of the cell preparations used in this study varied from 45 to 80 μg of chlorophyll per ml of assay medium.

Time-Course and Concentration Studies with Methazole, DCPMU, and DCPU

The assaying medium for all metabolic studies contained 2 ml of the cell preparation in a 25-ml Erlenmeyer flask, 0.1 ml of radioactive substrate containing 1 μCi of radioactivity, and 0.05 ml of the herbicide solution, making a final volume of 2.15 ml. The radioactive substrates included $NaH^{14}CO_3$ (sp. act. 44.4 mCi/mM) for photosynthesis, L-[U-^{14}C]-leucine (sp. act. 290 mCi/mM) for protein synthesis, [2-^{14}C]-uracil (sp. act. 55 mCi/mM) for ribonucleic acid (RNA) synthesis, and [1,2-^{14}C]-acetic acid (sp. act. 56.2 mCi/mM) for lipid synthesis. All radioactive substrates were purchased from ICN, Chemical and Radioisotope Division, Irvine, California. Analytical grade standards (100% pure) of methazole, DCPMU, and DCPU were provided generously by Velsicol Chemical Corp., Chicago, Illinois. Methazole, DCPMU, and DCPU were dissolved in ethanol and made up to volume with distilled water so that the final ethanol concentration was less than 1%. Herbicide concentrations of 0.1, 1, 10, and 100 μM were used in all assays. The Erlenmeyer flasks with the assay mixtures were sealed and placed in a shaking water bath at 25°C. The flasks were illuminated from above with a combination of fluorescent and incandescent lamps with 7.4 W m^{-2} at the level of the flasks. The assay mixtures were incubated for 30, 60, and 120 min. At the end of each incubation period, samples were collected and treated accordingly for each metabolic process. A detailed description of the specific procedures followed for the treatment of the collected samples has been reported elsewhere (Ashton et al. 1977, Hatzios and Penner 1980). The radioactivity of the treated samples was determined by radioassay with a Beckman LS-250 liquid scintillation spectrometer having an average counting efficiency of about 90%. Photosynthesis and protein, RNA, and lipid synthesis were calculated as counts per minute (cpm) of the [^{14}C] from the respective radioactive substrate incorporated into the cells per 100 μg of chlorophyll. The results were also calculated as percent inhibition caused by each concentration of methazole, DCPMU, and DCPU

as compared with the controls. All assays were repeated 3 times, and the data were analyzed for variance in a completely randomized design with a factorial arrangement of the treatments. Duncan's Multiple Range Test was used to separate the treatment means.

Results

Effects expressed by methazole, DCPMU, and DCPU on photosynthesis and protein, RNA, and lipid synthesis of enzymatically isolated leaf mesophyll cells of velvetleaf are presented in Tables 1–4. Photosynthesis as measured by the fixation of $^{14}CO_2$ from $NaH^{14}CO_3$ by the isolated velvetleaf cells was inhibited significantly and rapidly by DCPMU at concentrations of 1 μM or higher. The respective inhibitory percentages (71, 92, and 96%) caused by 1, 10, and 100 μM of DCPMU were observed at as early as 30 min of incubation time and remained unchanged up to 120 min, the maximum exposure time examined in this study (Table 1). DCPMU at 0.1 μM did not inhibit photosynthesis significantly at any incubation period examined. In contrast, methazole and DCPU inhibited photosynthesis significantly at all incubation periods examined only at the highest concentration of 100 μM (Table 1). Inhibition of photosynthesis by DCPU did not appear to be very strong and percentage values of inhibition did not increase with concomitant increases in incubation time. Inhibition of photosynthesis by methazole at 100 μM, however, was time-dependent and it increased from 33% at 30 min to 64% after 120 min of incubation time. Significant inhibition of photosynthesis of isolated leaf cells of velvetleaf was also caused by methazole at 10 μM but only after 120 min of incubation (Table 1). However, the percentage inhibition by methazole was relatively small (28%).

The incorporation of [^{14}C]-leucine into velvetleaf leaf cells was inhibited significantly by 100 μM of methazole, DCPMU, and DCPU at all incubation periods with the exception of methazole and DCPMU at the end of the 30 min incubation period, which were not inhibitory (Table 2). Significant inhibition of protein synthesis was also caused by both methazole and DCPMU at 10 μM but only at the 120 min exposure time (Table 2). The observed inhibition of protein synthesis by the highest concentration of all three chemicals (100 μM) appeared to be time-dependent, and the percent inhibition increased steadily with concomitant increases in incubation time (Table 2). After 120 min of incubation, the percent inhibitions of protein synthesis by DCPMU and DCPU were comparable and higher than that caused by methazole. The lower concentrations of methazole and DCPU (0.1 and 1.0 μM) caused slight stimulation of protein synthesis at the 30 and 60 min incubation periods (Table 2). However, these values were not statistically significant.

Data in Table 3 indicate that methazole, DCPMU, and DCPU, at 100 μM, significantly inhibited RNA synthesis of isolated velvetleaf cells at all incubation periods examined except for methazole at 30 min of incubation, which was not inhibitory. Significant inhibition of RNA synthesis was also observed with methazole and DCPU at 10 μM, but only at the 120 min incubation period. DCPMU, however, appeared to be a better inhibitor of RNA synthesis than either methazole or DCPU, since it inhibited RNA synthesis at concentrations as low as 1 μM following incubations of 60 and 120 min (Table 3). Inhibition of

Table 1. The effect of methazole, DCPMU, and DCPU on photosynthesis of isolated velvetleaf cells.[a]

Incubation time (min)	Herbicide concentration (μM)	Methazole		DCPMU		DCPU	
		$^{14}CO_2$-fixation (cpm/100 μg Chl)	Inhibition[b] (%)	$^{14}CO_2$-fixation (cpm/100 μg Chl)	Inhibition (%)	$^{14}CO_2$-fixation (cpm/100 μg Chl)	Inhibition (%)
30	0	54,816 g	0	59,288 ef	0	59,268 de	0
	0.1	54,802 g	0	53,279 f	10	55,905 de	6
	1	55,196 g	−1	17,275 h	71	55,518 de	6
	10	51,557 gh	6	4,445 ij	92	50,098 e	16
	100	36,914 i	33	2,430 j	96	37,739 f	36
60	0	95,542 d	0	102,256 c	0	100,981 c	0
	0.1	93,631 d	2	86,917 cd	15	97,951 c	3
	1	96,498 d	−1	28,632 g	72	99,971 c	1
	10	79,301 de	17	6,135 ij	94	93,912 c	7
	100	45,860 h	52	3,068 j	97	66,647 d	34
120	0	188,599 a	0	194,312 a	0	193,875 a	0
	0.1	173,511 ab	8	165,597 ab	15	188,058 a	3
	1	179,169 ab	5	62,180 e	68	184,181 a	5
	10	135,791 c	28	11,658 hi	94	186,120 a	4
	100	67,895 f	64	7,772 ij	96	126,018 b	35

[a] Means within columns followed by similar letters are not significantly different at the 5% level by Duncan's Multiple Range Test.

[b] A minus (−) sign in front of a percentage value indicates stimulation instead of inhibition.

Table 2. The effect of methazole, DCPMU, and DCPU on protein synthesis of isolated velvetleaf cells.[a]

Incubation time (min)	Herbicide concentration (μM)	Methazole [^{14}C]Leucine incorporated (cpm/100 μg Chl)	Methazole Inhibition[b] (%)	DCPMU [^{14}C]Leucine incorporated (cpm/100 μg Chl)	DCPMU Inhibition (%)	DCPU [^{14}C]Leucine incorporated (cpm/100 μg Chl)	DCPU Inhibition[b] (%)
30	0	25,180 ef	0	27,833 e	0	26,861 c	0
	0.1	27,228 ef	−8	26,806 e	4	28,180 c	−5
	1	28,136 ef	−12	26,670 e	4	29,421 c	−9
	10	22,380 fg	11	26,864 e	4	28,241 c	−5
	100	20,623 fg	12	20,375 ef	27	16,295 d	39
60	0	42,254 cd	0	48,010 d	0	49,004 b	0
	0.1	45,025 cd	−6	44,877 d	7	52,806 b	−8
	1	45,432 cd	−7	45,610 d	5	47,297 b	4
	10	35,809 de	15	44,512 d	7	45,820 b	7
	100	25,792 ef	39	30,013 e	38	23,774 c	52
120	0	77,754 a	0	79,246 a	0	78,317 a	0
	0.1	71,434 a	8	76,385 ab	4	76,626 a	2
	1	60,987 ab	22	64,389 abc	19	74,661 a	5
	10	52,850 bc	32	59,710 cd	25	70,074 a	11
	100	40,137 cd	48	29,665 e	63	29,705 c	62

[a] Means within columns followed by similar letters are not significantly different at the 5% level by Duncan's Multiple Range Test.
[b] A minus (−) sign in front of a percentage value indicates stimulation instead of inhibition.

Table 3. The effect of methazole, DCPMU, and DCPU on RNA synthesis of isolated velvetleaf cells.[a]

Incubation time (min)	Herbicide concentration (μM)	Methazole [^{14}C]Uracil incorporated (cpm/100 μg Chl)	Methazole Inhibition[b] (%)	DCPMU [^{14}C]Uracil incorporated (cpm/100 μg Chl)	DCPMU Inhibition (%)	DCPU [^{14}C]Uracil incorporated (cpm/100 μg Chl)	DCPU Inhibition[b] (%)
30	0	4,261 fg	0	3,804 cd	0	4,222 e	0
	0.1	4,002 fg	6	3,705 cd	3	4,350 e	−3
	1	4,324 fg	−1	2,782 de	27	4,230 e	0
	10	3,864 fg	9	2,781 de	27	4,055 e	4
	100	3,243 g	24	2,417 e	37	3,041 f	28
60	0	7,329 cd	0	6,723 b	0	6,843 d	0
	0.1	7,563 cd	−3	6,262 b	7	6,745 d	2
	1	7,398 cd	−1	4,185 cd	38	6,685 d	2
	10	6,393 d	13	4,150 cd	38	6,217 d	9
	100	4,887 ef	33	4,162 cd	38	4,380 e	36
120	0	13,126 a	0	12,193 a	0	12,294 a	0
	0.1	11,207 ab	15	11,190 a	8	11,270 ab	8
	1	11,961 ab	9	6,114 b	50	9,994 abc	19
	10	8,271 c	37	6,628 b	46	9,159 c	26
	100	6,109 de	54	5,569 bc	54	6,843 d	44

[a] Means within columns followed by similar letters are not significantly different at the 5% level by Duncan's Multiple Range Test.

[b] A minus (−) sign in front of a percentage value indicates stimulation instead of inhibition.

Table 4. The effect of methazole, DCPMU, and DCPU on lipid synthesis of isolated velvetleaf cells.[a]

Incubation time (min)	Herbicide concentration (μM)	Methazole [^{14}C]Acetate incorporated (cpm/100 μg Chl)	Methazole Inhibition[b] (%)	DCPMU [^{14}C]Acetate incorporated (cpm/100 μg Chl)	DCPMU Inhibition (%)	DCPU [^{14}C]Acetate incorporated (cpm/100 μg Chl)	DCPU Inhibition[b] (%)
30	0	96,448 de	0	98,098 c	0	95,597 de	0
	0.1	108,264 de	−12	89,049 c	9	97,321 de	−1
	1	81,820 e	15	95,760 c	3	93,769 de	2
	10	74,784 e	23	29,305 d	70	80,160 de	16
	100	74,249 e	23	6,101 e	94	63,134 f	34
60	0	194,240 c	0	189,944 b	0	190,481 bc	0
	0.1	214,608 c	−10	178,906 b	6	191,909 bc	0
	1	159,392 cd	18	198,872 b	− 5	154,201 bcd	19
	10	121,170 de	38	57,496 cd	70	136,528 de	28
	100	116,717 de	40	9,961 e	95	135,914 de	29
120	0	347,801 ab	0	358,287 a	0	358,787 a	0
	0.1	385,849 a	−10	390,834 a	− 9	345,078 a	4
	1	286,183 b	18	343,385 a	4	341,419 a	5
	10	210,768 c	26	89,874 c	75	224,092 b	38
	100	199,473 c	43	12,179 de	97	215,765 bc	40

[a] Means within columns followed by similar letters are not significantly different at the 5% level by Duncan's Multiple Range Test.

[b] A minus (−) sign in front of a percentage value indicates stimulation instead of inhibition.

RNA synthesis by all three chemicals appeared to be somewhat time-dependent, since percent inhibition increased slightly with increases in incubation time.

Significant inhibition of lipid synthesis as measured by the incorporation of [^{14}C]-acetic acid into isolated velvetleaf leaf cells occurred with all three chemicals tested at the 10 and 100 μM concentrations after a 60 and 120 min incubation period (Table 4). In addition, DCPMU at 10 and 100 μM and DCPU at 100 μM were also inhibitory after 30 min incubation. The percent inhibition of lipid synthesis caused by DCPMU was much higher than that caused by either methazole or DCPU. Furthermore, inhibition of lipid synthesis by DCPMU was very rapid, reaching maximum levels after 30 min incubation. Inhibition of lipid synthesis caused by either methazole or DCPU appeared to be dependent on incubation time. Methazole at 0.1 μM caused slight stimulation of lipid synthesis in velvetleaf leaf cells but, again, this stimulation was not statistically important (Table 4).

Discussion

The results of the present study revealed that DCPMU was the most effective inhibitor of the four metabolic processes examined in isolated leaf cells of velvetleaf. The lowest concentration of DCPMU that inhibited any of the metabolic processes studied was 1 μM. After 120 min incubation, photosynthesis and RNA synthesis were inhibited 68% and 50%, respectively. At the highest concentration (100 μM) and maximum exposure time (120 min), the four metabolic processes were inhibited by DCPMU as follows: photosynthesis, 96%; protein synthesis, 63%; RNA synthesis, 54%; and lipid synthesis, 97%. Although photosynthesis appeared to be the most sensitive site of inhibition by DCPMU, protein, RNA, and lipid synthesis could also be involved in the ultimate phytotoxic action of this chemical. The potential of DCPMU as an inhibitor of the Hill reaction of photosynthesis in isolated chloroplasts has been reported by a number of investigators (Brockman 1975, Good 1961, Suzuki and Casida 1980, 1981). In addition, other substituted phenylurea herbicides such as monuron [3-(*p*-chlorophenyl)-1,1-dimethylurea] and diuron [3-(3,4-dichlorophenyl)-1,1-dimethylurea], which are well-known photosynthetic inhibitors, have also been reported to interfere with protein, RNA, and lipid synthesis in treated plant tissues (Ashton et al. 1977, Moreland 1980). However, the effects of photosynthesis-inhibiting herbicides on these physiological processes could be either direct or indirect, resulting from the primary action of these chemicals on photosynthesis (Moreland 1980). Photosynthesis provides most of the cellular energy (ATP) needed for the biosynthetic pathways involved in protein, RNA, and lipid synthesis of plants. Inhibition of protein and RNA synthesis by DCPMU was progressive, and the effects of DCPMU on protein, RNA, and lipid synthesis were generally caused by concentrations of this phytotoxicant higher than those that inhibited photosynthesis. This suggests that the effects of DCPMU on physiological processes other than photosynthesis are indirect and are brought about by its direct effect on photosynthesis. However, further studies are needed before a direct effect of DCPMU on these processes could be excluded.

The lowest concentration of methazole that significantly inhibited any of the four metabolic processes studied was 10 μM. At this concentration, methazole inhibited photosynthesis 28%, protein synthesis 32%, RNA synthesis 37% and lipid synthesis 26% at the maximum exposure time of 120 min. At the highest concentration (100 μM) and maximum incubation time (120 min), these processes were inhibited by methazole as follows: photosynthesis, 64%; protein synthesis, 48%; RNA synthesis, 54%; and lipid synthesis, 43%. Inhibition of the four metabolic processes by methazole was also time-dependent, increasing in magnitude with concomitant increases in incubation time. The progressive character of the interferences of methazole with photosynthesis and protein, RNA, and lipid synthesis would seem to indicate that the observed inhibitions at 120 min of incubation were not caused by methazole itself but by DCPMU formed through the metabolism of methazole by the isolated leaf cells of velvetleaf. Such a possibility is supported by a previous study of Butts and Foy (1974), who reported that leaf discs of prickly sida (*Sida spinosa* L.) and cotton (*Gossypium hirsutum* L.) were able to convert 38% and 28% of the radioactivity associated with methazole to DCPMU, respectively, after being incubated with ^{14}C-methazole for 90 min.

The lowest concentration of DCPU that appreciably inhibited any of the four metabolic processes examined in the present study was 10 μM, and it involved only a 26% inhibition of RNA synthesis and a 38% inhibition of lipid synthesis at the maximum incubation of 120 min. At the highest concentration (100 μM) and maximum exposure (120 min), the percentages of DCPU inhibition of photosynthesis and protein, RNA, and lipid synthesis were 35%, 62%, 44%, and 40%, respectively. Thus, with the exception of protein synthesis, inhibition of any metabolic process by the highest concentration of DCPU was slight and less than 50%. Inhibition of protein and RNA synthesis by DCPU appear to be time-dependent, while the effects of DCPU on photosynthesis and lipid synthesis were less affected by increases in incubation time.

Data presented in this study are supportive of the proposal of previous studies that methazole is a pro-herbicide and its conversion to DCPMU represents an example of herbicide bioactivation through metabolism by higher plants (Brockman 1975, Suzuki and Casida 1981). Additional examples of bioactivation of other herbicides are also known and they have been reviewed (Hatzios and Penner 1982). In general, DCPU was less active than the parent herbicide methazole or DCPMU, and it appeared to be a terminal metabolite with reduced phytotoxicity.

Acknowledgments. I express my sincere appreciation to Velsicol Chemical Corporation, Chicago, Illinois (USA) for providing the analytical standards of methazole, DCPMU, and DCPU and partial financial support for completing this study. Gratitude is also expressed to Mrs. Celestia M. Howe for her able technical assistance throughout this study.

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