Absorption, Translocation, and Metabolism of Radioactive 3-(*p*-Chlorophenyl)-1,1-dimethylurea (CMU) by Bean Plants

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A study has been made of the absorption and translocation of carbonyl-C^{14}–labeled 3-(*p*-chlorophenyl)-1,1-dimethylurea (CMU) by bean plants. When this chemical is applied to the foliage of bean plants, it is quickly absorbed and translocated throughout the entire leaf. Translocation to the other parts of the plant is much slower. By means of paper chromatography, two major radioactive compounds have been found in 80% ethyl alcohol extracts of leaves from bean plants treated with radioactive CMU. One of the compounds was identified as unchanged CMU and the other was an unknown radioactive CMU complex. The concentration of CMU in the leaf extract decreases with time, while a corresponding increase occurs in the concentration of the CMU complex.

THE COMPOUND 3-(*p*-chlorophenyl)-1,1-dimethylurea (CMU) was demonstrated by Bucha and Todd (*1*) to be active as a plant growth inhibitor and is recognized as a promising pre-emergence herbicide and soil sterilant. It is one of the first organic compounds to possess a long residual activity in soil.

CMU is very effective in killing many species of plants. The initial symptom is chlorosis at the tips of leaves, beginning with the older leaves. This is followed by the retardation of growth and ends in the death of the plant, if a lethal dosage is used. In general practice, the chemical is applied to both the foliage and the soil. Soil applications are made for pre-emergence weed control. It has been suggested by several workers (*1*, *5*) that CMU is absorbed through the root system, from which it is translocated upward to the leaves, as well as being absorbed by the foliage.

It was the purpose of this investigation to study the extent of absorption of carbonyl-C^{14}–labeled CMU and the translocation of carbon-14 in bean plants.

Experimental Methods

Culture of Plants Bean plants (*Phaseolus vulgaris*, var. Black Valentine) were grown in flats containing Chehalis sandy loam soil under greenhouse conditions. A 95% ethyl alcohol solution containing 0.1% radioactive 3-(*p*-chlorophenyl)-1,1-dimethylurea and 1% Tween-20 (weight/volume) was used for treatment. The plants were treated when the primary leaves were almost fully expanded and the terminal buds were still very small. The solution was applied quantitatively by means of a microsyringe to each plant along the midrib of one primary leaf. After harvesting, all plants were sectioned and homogenized with 80% alcohol (*2*).

Methods of Study Before the study was made with radioactive CMU, preliminary studies using nonradioactive CMU were carried out. Forty-eight bean plants were divided into twelve groups of four plants each. All groups were treated at the same time with various amounts of nonradioactive CMU in order to determine the proper dosage of material to be used in the study with the radioactive chemical. The study revealed that there was no toxic effect on bean plants if a dosage of less than 20 γ per plant was used. With a dosage of 40 to 60 γ per plant, chlorosis of the treated leaves was generally observed within 3 or 4 days, but the plants recovered from the effect and the new leaves started to grow after 10 to 14 days. When the dosage was greater than 200 γ per plant, necrosis was generally noted first in the treated leaves, next in the nontreated leaves, followed by the death of the plants. It was decided then that more information might be obtained if two levels of dosage in foliage application were used in the study with radioactive CMU.

Experiment 1. Twenty bean plants were treated with 19 γ of radioactive CMU per plant in the manner described. Groups of five plants each were harvested 2, 4, 24, and 72 hours after treatment. One plant from each group was used for making an autoradiogram and the remaining plants were divided into leaf, petiole, terminal bud, first internode, hypocotyl, and root sections. Each section was pooled, weighed, and homog-

Table I. Absorption and Translocation of Carbon-14 Applied as Radioactive CMU in Bean Plants

(Each plant treated with 19 γ of CMU)

	2 Hours		4 Hours		1 Day		3 Days	
	Total activity, c.p.m.	% of amt. applied	Total activity, c.p.m.	% of amt. applied	Total activity, c.p.m.	% of amt. applied	Total activity, c.p.m.	% of amt. applied
No. of plants used	4		4		4		3	
Total activity applied	165,000		165,000		165,000		123,500	
Activity recovered								
Treated leaves	160,000	96.97	151,000	91.52	151,500	91.82	121,000	97.98
Treated petioles	0	0	12,600	7.64	600	0.36	85	0.05
Untreated leaves	0	0	0	0	0	0	0	0
Untreated petioles	0	0	0	0	0	0	0	0
Terminal buds	0	0	3,750	2.27	0	0	120	0.07
First internodes	0	0	180	0.11	0	0	0	0
Hypocotyls	165	0.10	0	0	100	0.06	0	0
Roots	200	0.12	300	0.18	180	0.11	230	0.14

Table II. Absorption and Translocation of Carbon-14 Applied as Radioactive CMU in Bean Plants

(Each plant treated with 50 γ of CMU)

	1 Hour		1 Day		2 Days		4 Days		8 Days		12 Days	
	Total activity, c.p.m.	% of amt. applied	Total activity, c.p.m.	% of amt. applied	Total activity, c.p.m.	% of amt. applied	Total activity, c.p.m.	% of amt. applied	Total activity, c.p.m.	% of amt. applied	Total activity, c.p.m.	% of amt. applied
No. of plants used	4		4		4		4		4		4	
Total activity applied	433,200		433,200		433,200		433,200		433,200		433,200	
Activity recovered												
Treated leaves	387,050	89.35	...		358,650	82.79	330,300	76.25	316,670	73.10	326,880	75.46
Treated petioles	10,500	2.42	7,150	1.65	8,400	1.94	4,180	0.97	375	0.09	1,650	0.38
Untreated leaves	500	0.12	1,425	0.33	6,800	1.57	24,200	5.59	750	0.17	1,500	0.35
Untreated petioles	175	0.04	600	0.14	2,230	0.52	1,425	0.33	125	0.03	425	0.10
Stems	550	0.13	4,250	0.98	14,450	3.34	33,700	7.78	1,250	0.29	700	0.16
Roots	125	0.03	300	0.07	1,100	0.25	225	0.05	150	0.03	400	0.09

enized with 80% alcohol. The radioactivity of the alcohol extracts was measured by a windowless gas flow Geiger counter using a direct plating technique.

Experiment 2. A dosage of 50 γ of radioactive CMU per plant was used. Groups of five plants each were harvested 1 hour, and 1, 2, 4, 8, and 12 days after treatment. Four plants from each group were sectioned and homogenized separately with 80% alcohol as in Experiment 1.

Paper Chromatography

The alcoholic extracts of treated leaves from Experiment 2 were applied separately to a spot about 7 cm. from the end of strips of Whatman No. 1 filter paper (1 × 22 inches) by means of a fine eye dropper. The chromatograms were developed with a mixture of 1-butanol, acetic acid, and water (4:1:1 volume/volume) for 18 hours. A descending chromatographic technique was used. After developing, the strips were dried and cut into 1-cm. sections, starting from the original spot. The radioactivity of each section was determined directly. The relative activities of the radioactive spots on the chromatograms were calculated according to the method described in a previous paper (*2*).

Results and Discussion

The results from Experiment 1 are shown in Table I. In all four samples, more than 90% of the radioactivity remained in the treated leaves. Only a small amount of carbon-14 was translocated to the other parts of the plants. This observation indicated that the movement of CMU from the foliage to the stem is rather slow.

When a larger amount of radioactive CMU per plant was used in Experiment 2, slightly more radioactivity was found on plant parts other than the treated leaves, as shown in Table II. This slight discrepancy might result from the increased temperature and total daylight hours of the latter experiment. These factors could also account for a more rapid absorption and translocation of CMU, as the rate of absorption and translocation of 2,4-D is influenced by light and temperature (*3*, *6*, *7*). The autoradiograms of bean plants harvested 1, 2, and 4 hours after CMU treatment from Experiments 1 and 2 revealed that the absorption of this chemical and its translocation through the entire treated leaf were rapid. The chemical appeared to be translocated through the veins. The radioactivity in the petiole of the treated leaf reached the maximum within 1 hour after treatment, while in the stem and in the untreated leaf, the maximum concentrations did not occur until the fourth day. Excluding the treated leaf, the recovery of radioactivity from the plant parts of the 8- and 12-day samples was less than 1%, indicating a great decrease of CMU in them. This finding suggests that the chemical may be metabolized and subsequently released as radioactive carbon dioxide. The radioactivity in the treated leaves remained unchanged after 4 days. This may be explained by the fact that chlorosis was observed in the treated leaves of all plants 2 to 4 days after treatment. The leaves, after suffering a severe injury, could not further translocate or metabolize the chemical remaining in them.

Figure 1 is the autoradiogram of a bean plant harvested 2 days after CMU treatment. Chlorosis was observed on the treated leaf of this plant. The spots which show higher concentration of radioactivity coincided with necrotic spots in the treated leaf.

The results from paper chromatographic study of 80% alcoholic extract of treated leaf are presented in Table III. Only two compounds containing radioactive carbon were noted. One is unchanged CMU, which has an R_f value between 0.84 and 0.87 in butanol–acetic acid–water solvent. The identity of the other radioactive compound, which has an R_f value between 0.62 and 0.66 in the same solvent system, is not yet known. In a time course study the concentration of CMU decreased while the concentration of the other radioactive compound increased. No other noticeable radioactive compounds were formed during the period of 12 days. The radioactive unknown compound has been hydrolyzed with 2*N* hydrochloric acid. Chromatographic analysis of the hydrolyzate indicated that 91% of the CMU in the complex was released after 2 hours of refluxing. This would suggest that this unknown compound is a complex of CMU, which may arise from the detoxification process.

Lowen and Baker (*4*) have shown that digesting CMU with dilute acid for 2 hours results in significant hydrolysis of the molecule. The breakdown products are *p*-chloroaniline, dimethylamine, and carbon dioxide. It would be expected that hydrolysis of the CMU complex

Figure 1. Autoradiogram of bean plant harvested 2 days after 50 γ radioactive CMU treatment

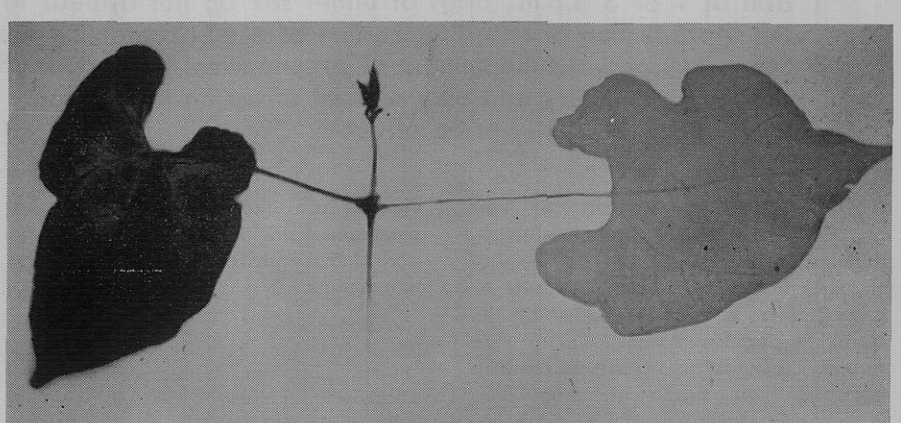

Table III. Distribution of Major Radioactive Compounds in 80% Ethyl Alcohol Extract of Bean Leaves

(After treatment with 50 γ of carbonyl-C^{14}–labeled CMU. Plants harvested after varying intervals)

Harvested, Days after Treatment	R_f 0.62–0.66, CMU Complex, %	R_f 0.84–0.87, Free CMU, %
1 hour	0	100
1	5	93
2	11	87
4	13	85
8	19	80
12	19	81

might result in a similar degradation. However, only two compounds containing carbon-14 in the acid hydrolyzate were revealed by the paper chromatographic technique when aqueous phenol or 1-butanol, propionic acid, and water were used as solvents. Therefore, it is believed that the cleavage of CMU-complex linkage must take place first during the hydrolysis, and subsequently the free carbonyl-C^{14}-CMU breakdown to form nonradioactive *p*-chloroaniline, dimethylamine, and radioactive carbon dioxide, which are not detected by paper chromatography. At the later stages of this experiment, the increase in concentration of CMU complex was small, indicating a general reduction and destruction of the metabolic activity. In a study with radioactive 2,4-D, Jaworski and Butts demonstrated that approximately 60% of carbon-14 was in the form of the 2,4-D complex 4 days after treatment (*2*). In the study with CMU, less than 20% of radioactive CMU has undergone a biochemical reaction. These results reveal the difference in rate of reaction between plant substrates and an exogenous chemical.

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PESTICIDE TOXICITY

Toxicity of Certain Chlorinated Hydrocarbon Insecticides for Laboratory Animals, with Special Reference to Aldrin and Dieldrin

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The immediate toxicity of four polychlorinated dimethanonaphthalenes—aldrin, isodrin, dieldrin, and endrin—compared on the basis of oral administration to rats or rabbits or as applied and maintained upon the skin of rabbits, depends more directly upon their spatial configuration than their empirical composition. Repetitive applications of aldrin, dieldrin, or DDT upon the skin of rabbits exerted toxic effects decreasingly, according to the use or type of a vehicle, in the following order: in Ultrasene, in a vegetable oil (aldrin and DDT in olive oil and dieldrin in peanut oil), and as dry powders (no vehicle). When fed for 2 years to rats of either sex at levels of 2.5, 12.5, or 25.0 p.p.m., aldrin, dieldrin, and DDT do not appear to shorten the lives of the animals, the rate of mortality among the test groups being comparable statistically to that in corresponding control groups. The rates of growth of the test groups were equal to or in excess of that of the controls. The weights of the livers of the test rats, in relation to their body weights, were somewhat on the high side. Dogs are more susceptible than rats to the toxic effects of aldrin or dieldrin. In prolonged periods of feeding on diets containing aldrin or dieldrin at 1 or 3 p.p.m., dogs of either sex do not appear to be affected adversely. In the reproduction of rats, the feeding of a diet containing dieldrin in the concentration of 2.5 p.p.m. reduced the number of pregnancies, but had no effect upon the number of offspring per delivery and only a slight effect on the mortality of the suckling rats. At this level, aldrin had little or no effect.

THE IMMEDIATE TOXICITY of four polychlorinated dimethanonaphthalenes (aldrin, isodrin, dieldrin, and endrin), when given orally to rats or rabbits, is more closely related to the spatial configuration than to the empirical composition. Aldrin and isodrin have the same empirical composition and so do their respective epoxides, dieldrin and endrin, but in spatial configuration aldrin and dieldrin, and isodrin and endrin, form closely related pairs (Figure 1).

Aldrin (Compound 118 or Octalene) is the coined name for the insecticidal product containing not less than 95% of 1,2,3,-4,10,10 - hexachloro - 1,4,4a,5,8,8a - hexahydro - 1,4 - *endo,exo* - 5,8 - dimethanonaphthalene (commonly referred to as HHDN) and not more than 5% of related compounds.

Isodrin (Compound 711) is the coined name for 1,2,3,4,10,10-hexachloro-1,4,4a,-5,8,8a - hexahydro - 1,4 - *endo,endo* - 5,8-dimethanonaphthalene.

Dieldrin (Compound 497 or Octalox) is the coined name for the insecticidal product containing not less than 85% of 1,2,3,-