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Metabolism of Isouron [3-(5-*tert*-Butyl-3-isoxazolyl)-1,1-dimethylurea]: Rapid Conversion of the Herbicide to Less Phytotoxic Metabolites in a Resistant Plant, Sugarcane

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The metabolism of isouron [3-(5-*tert*-butyl-3-isoxazolyl)-1,1-dimethylurea] in a resistant plant, sugarcane, and inhibitory activities of isouron and its metabolites on photosynthetic O_2 evolution in *Chlorella* were investigated. [^{14}C]Isouron was applied in a 10^{-4} M solution to the plants through the roots for 4 h. After a 168-h metabolic period unchanged isouron comprised 22.7% of the total radioactivity found in the plants, and the following metabolites were formed by N-demethylation and hydroxylation of *tert*-butyl group: monomethylurea derivative [3-(5-*tert*-butyl-3-isoxazolyl)-1-methylurea] (6.4%); (hydroxymethyl)urea derivative [3-(5-*tert*-butyl-3-isoxazolyl)-1-(hydroxymethyl)urea] (2.4%); urea derivative [3-(5-*tert*-butyl-3-isoxazolyl)urea] (2.0%); (hydroxy-*tert*-butyl)dimethylurea derivative [3-[5-(1,1-dimethyl-2-hydroxyethyl)-3-isoxazolyl]-1,1-dimethylurea] (0.7%); (hydroxy-*tert*-butyl)monomethylurea derivative [3-[5-(1,1-dimethyl-2-hydroxyethyl)-3-isoxazolyl]-1-methylurea] (1.5%); (hydroxy-*tert*-butyl)urea derivative [3-[5-(1,1-dimethyl-2-hydroxyethyl)-3-isoxazolyl]urea] (14.3%). Monomethylurea derivative had an inhibitory activity about half that of isouron on photosynthetic O_2 evolution, but the other metabolites had no significant activities. Furthermore, isouron was converted to more polar metabolites, which were tentatively identified as O-glucosides of 3-(5-*tert*-butyl-3-isoxazolyl)-1-(hydroxymethyl)-1-methylurea (10.5%) and (hydroxymethyl)urea (9.1%) and (hydroxy-*tert*-butyl)urea (4.2%) derivatives.

INTRODUCTION

Isouron is an isoxazolylurea herbicide for controlling total vegetation in noncroplands (Ito et al., 1979) and for selective weed control in sugarcane fields (Yukinaga et al., 1979a). As isouron inhibits the Hill reaction in isolated spinach chloroplasts, the primary site of its action has been suggested to be in the photosynthetic electron-transport system (Yukinaga et al., 1979b). We previously reported that the major metabolic pathways of the herbicide in a susceptible plant, bean, were N-demethylation and hydroxylation of the *tert*-butyl group, followed by glucose conjugation (Ishizuka et al., 1982a). It was found that, in bean plants, the rate of isouron metabolism was slow; much of the isouron absorbed by the plant remained unchanged, and the major metabolite was the first demethylation product, the monomethylurea derivative. Since in many cases differences in the rate of metabolism of a herbicide among different plant species cause a selective action of the chemical (Jensen, 1982; Jacobson and Shimabukuro, 1984), a relative insensitivity of sugarcane plant to isouron may be derived from its rapid conversion in the plant to metabolites that are less active on photosynthetic electron transport.

The purpose of this study, therefore, is to establish the pathway and rate of isouron metabolism in a resistant plant, sugarcane, and to determine the inhibitory activities of isouron and its metabolites on photosynthetic O_2 evolution in *Chlorella*. A preliminary account of this work has been given earlier (Ishizuka et al., 1982b).

MATERIALS AND METHODS

Chemicals. Isouron and its related compounds were synthesized at Shionogi Research Laboratories, Fukushima-ku, Osaka, Japan. [^{14}C]Isouron labeled at the 5-position of the isoxazole ring was also prepared at the laboratories and had a specific activity of 8.28 mCi/mmol. Its radiochemical purity as determined by thin-layer chromatography (TLC) was greater than 98.5%.

Plant Materials. Vegetative stalk propagule pieces of sugarcane (*Saccharum officinarum* L. cv. NCo-310) were surface sterilized with a 0.1% (w/v) sodium hypochlorite solution for 15 min, washed with tap water, and planted in moist sand. The seedlings sprouted were grown for 3

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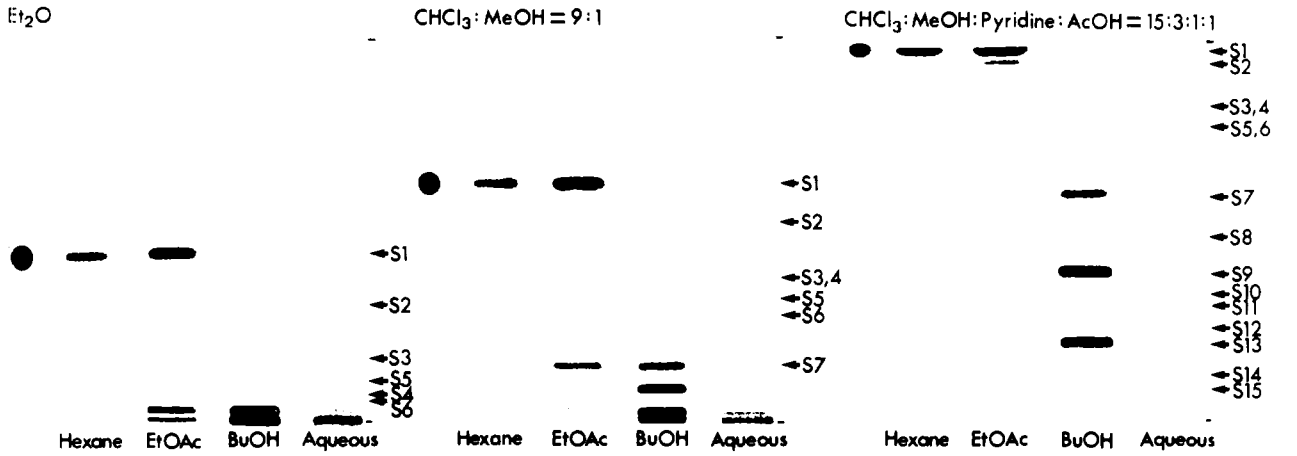

Figure 1. Autoradiography of [^{14}C]isouron and its metabolites. Hexane, ethyl acetate, butanol, and aqueous fractions from the plants grown for 168 h after a 4-h root application of [^{14}C]isouron were chromatographed by TLC with ethyl ether, $CHCl_3$–MeOH (9:1, v/v), and $CHCl_3$–MeOH–pyridine–AcOH (15:3:1:1, v/v). Authentic [^{14}C]isouron was cochromatographed as a standard (left lane of each plate).

weeks in a greenhouse. Then, they were transferred to water culture in a Hoagland's nutrient solution (Hoagland and Arnon, 1938) and allowed to grow for 3 days prior to herbicide treatment to the root. The nutrient solution was aerated for 30 min at 6-h intervals during the growth period.

Application of [^{14}C]Isouron. Lots of five uniform seedlings were selected. Each lot was supplied with [^{14}C]isouron through the roots at a 10^{-4} M concentration in a 100-mL beaker under fluorescent lights (2.8×10^4 1x). After 4 h the plants were removed from the beaker, and their roots were rinsed three times with distilled water. The plants then were transferred to a fresh nutrient solution and grown under a photoperiod of 12-h light (2.8×10^4 1x) and 12-h dark at 29 °C. The treatment and rinse solutions were combined, and the radioactivity was determined. The uptake of [^{14}C]isouron by the plants was estimated by determining the radioactivities of the treatment solutions before and after application to the roots. At 0, 48, and 168 h after the treatment, the plants were removed from the nutrient solution and extracted after their roots were rinsed thoroughly with distilled water.

Extraction of Radioactive Metabolites. Radioactive metabolites were extracted from the plants, which were free of stalk propagule pieces and fractionated by solvents according to the method previously described for bean plants (Ishizuka et al., 1982a). Metabolites were separated into *n*-hexane, ethyl acetate, and 1-butanol solubles and the aqueous fraction.

Solvent Systems Used for TLC and HPTLC. For separation and identification of metabolites by TLC and/or HPTLC, combinations of the following solvent systems were used: solvent system I, diethyl ether; solvent system II, acetone; solvent system III, $CHCl_3$–MeOH (9:1, v/v); solvent system IV, $CHCl_3$–MeOH–pyridine–AcOH (15:3:1:1, v/v/v/v); solvent system V, MeOH–H_2O (7:3, v/v).

Time Course Study. After determination of the total radioactivity in each fraction, aliquots of the individual fractions at different harvest times were dried under reduced pressure at 40 °C. The residue was redissolved in a small amount of a solvent corresponding to solubility of the fraction and spotted onto a precoated 0.25-mm-thick silica gel 60 F_{254} TLC plate (Merck). The plates were developed with solvent systems I, III, and IV. The radioactive areas were located by autoradiography on Fuji X-ray film, IX-150. The radioactive compounds were designated as S1–S15 in a descending order of their R_f values on the TLC (Figure 1). Each area was scraped separately from the plate and radioassayed.

Isolation and Identification of Radioactive Metabolites. [^{14}C]Isouron and its metabolites were separated on TLC plates with three solvent systems as described above, and each radioactive component was further purified by TLC using the same solvent systems. Portions of the purified compounds S1–S7 were treated overnight with acetic anhydride–pyridine at room temperature. The isolated compounds (S1–S7) and their acetyl derivatives were identified by cochromatography by high-performance thin-layer chromatography (HPTLC) with silica gel 60 F_{254} or RP-8 F_{254s} (Merck) with the authentic specimens of presumed compounds. The polar compounds (S8–S15) were hydrolyzed with β-glucosidase (from sweet almonds, Boehringer) as described previously (Ishizuka et al., 1982a), and their hydrolytic products were identified by HPTLC.

Radioactive Analysis. Measurements of radioactivity in the treatment solutions, extracts, tissue debris, TLC plates, etc., were done by the methods previously described (Ishizuka et al., 1982a).

Application of Unlabeled Isouron. The seedlings were cultured for 2 days in 10^{-3} M isouron solution under the greenhouse conditions; then, the culture medium was changed to fresh Hoagland's nutrient solution and allowed to grow for 2 weeks. Nutrient solution was changed every 2 days.

Purification and Identification of Nonradioactive Metabolites. The nonradioactive metabolites were extracted with methanol, and methanol solubles were successively fractionated into *n*-hexane, ethyl acetate, 1-butanol, and aqueous fractions. The metabolites in the organic solvent fractions were further purified by series of column chromatography and TLC as illustrated in Figure 2. Mass spectra were obtained with Hitachi M-68 mass spectrometer equipped with a Hitachi Datalyzer, Model 002B. ^{1}H NMR spectra were recorded on a Varian XL-200 spectrometer; chemical shifts are reported in δ values downfield from internal tetramethylsilane.

Photosynthetic O_2 Evolution in *Chlorella*. Photosynthetic O_2-evolution assay was carried out in a 25-mL reaction cell. *Chlorella ellipsoidea* cells at a concentration of 2.4×10^6 cells mL^{-1} were suspended in 0.1 M phosphate buffer (pH 6.0) containing 0.1 M glucose and incubated at 25 °C. Changes in O_2 concentration of the suspension were measured with a Clark-type electrode in light (4×10^3 1x) and dark. Photosynthetic O_2 evolution was esti-

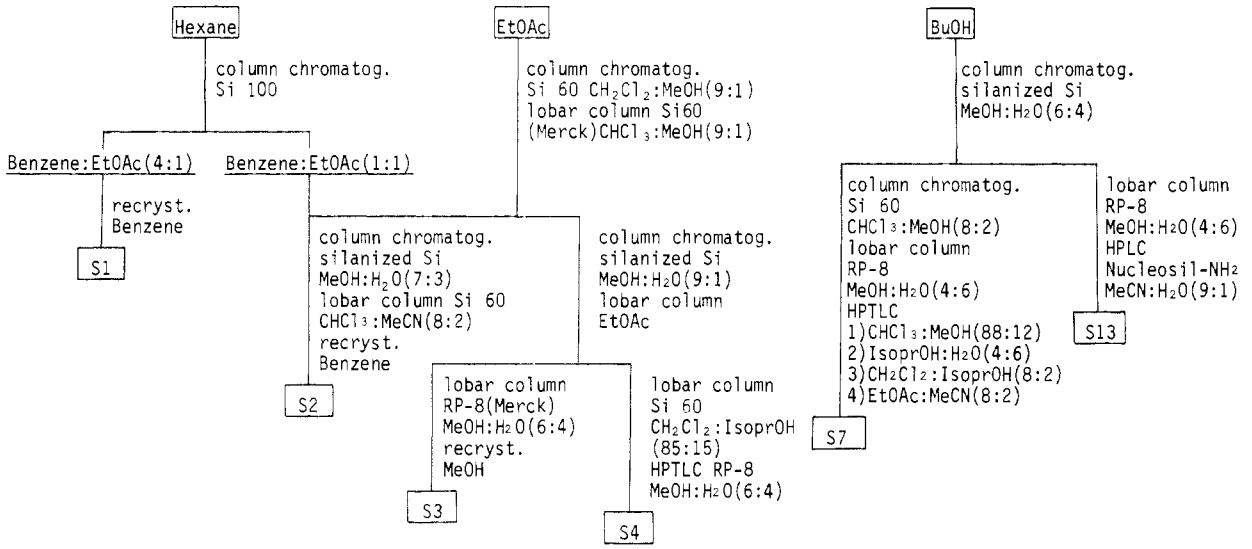

Figure 2. Outline of the procedures for isolation of isouron and its metabolites from sugarcane seedlings.

Table I. Recovery of Radioactivity of [^{14}C]Isouron Taken Up by Sugarcane Seedlings

	rec of radioact, × 10^4 dpm: h after end of treatment		
	0	48	168
abs in plants (*a*)	1181.2	1213.4	1386.6
	1127.6	1214.4	1394.9
	1237.6	1213.8	1272.3
leakage to med (*b*)		580.4	691.3
		599.8	690.9
		620.2	669.1
extracts and tissue	506.8	384.1	421.2
debris (*c*)	467.2	345.4	454.7
	457.3	383.3	363.6
stalk propagule pieces	686.8	179.9	244.4
(*d*)	719.0	201.7	197.7
	732.5	198.5	210.6
total rec, %	101.0	94.3	97.9
$[(b + c + d)/a \times 100]$	105.2	94.4	96.3
	96.1	99.0	97.7
mean ± std error	100.8 ± 2.6	95.9 ± 1.6	97.3 ± 0.5

mated by difference between the light and dark rates. Inhibitory activity was expressed as the molar concentration required to reduce the photosynthetic O_2 evolution by 50% (I_{50}). Isouron and its metabolites were dissolved in acetone (0.1% final concentration) and the resultant solutions added to the reaction mixture.

RESULTS

Recovery and Distribution of Radioactivity in the Extracted Fractions. The radioactivity absorbed by the plants was recovered almost completely in the extracts, tissue debris, stalk propagule pieces, and leakage. Even after 168 h the recovery was 97% (Table I). The radioactivities incorporated into the hexane and ethyl acetate fractions decreased rapidly with time during the growth period, while corresponding increases occurred in the butanol, aqueous, and tissue debris fractions (Figure 3). Radioactivity in the butanol fraction accounted for about 38% of the recovered total radioactivity at 168 h.

Identification of Metabolites. The TLC autoradiograms of the hexane, ethyl acetate, 1-butanol, and aqueous fractions in solvent systems I, III, and IV gave at least 15 radioactive spots (Figure 1). The R_f values in four different solvent systems of the purified S1–S4, S6, and S7 coincided with isouron, the monomethylurea derivative, the urea derivative, the (hydroxy-*tert*-butyl)dimethylurea derivative, the (hydroxy-*tert*-butyl)monomethylurea derivative,

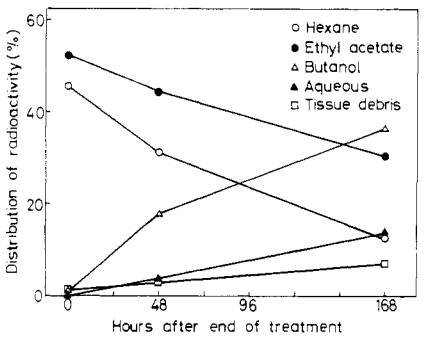

Figure 3. Changes in the distribution of radioactivity in separated fractions of sugarcane seedlings treated with [^{14}C]isouron. The percentages are based on the total radioactivity found in the plants.

Table II. R_f Values of Isouron and Its Metabolites in Various Solvent Systems

synth ref compd	R_f values in solv syst[a] I[b]	II[b]	III[b]	IV[b]	V[c]	corresp compd
isouron	0.23	0.60	0.64		0.44	S1
monomethylurea	0.14	0.55	0.54		0.43	S2
urea	0.06	0.49	0.34		0.53	S3
(hydroxy-*tert*-butyl)-dimethylurea	0.03	0.49	0.36		0.67	S4
(hydroxymethyl)urea	0.04	0.46	0.30		0.67	S5
(hydroxy-*tert*-butyl)-monomethylurea	0.02	0.50	0.26		0.68	S6
(hydroxy-*tert*-butyl)urea		0.39	0.14	0.47	0.76	S7

[a] Solvent systems: I, ethyl ether; II, acetone; III, $CHCl_3$–MeOH (9:1); IV, $CHCl_3$–MeOH–pyridine–AcOH (15:3:1:1); V, MeOH–H_2O (7:3). [b] HPLC plate silica gel 60 F_{254}. [c] HPLC plate RP-8 F_{254s}.

and the (hydroxy-*tert*-butyl)urea derivative, respectively (Table II). Compounds S4, S6, and S7 were acetylated, but S1–S3 were not. Acetylated S4, S6, and S7 were identical with the acetates of (hydroxy-*tert*-butyl)dimethylurea, (hydroxy-*tert*-butyl)monomethylurea, and (hydroxy-*tert*-butyl)urea derivatives, respectively, on TLC. On the basis of these results, S1–S4 and S6 were identified as unaltered isouron, monomethylurea, urea, (hydroxy-*tert*-butyl)dimethylurea, and (hydroxy-*tert*-butyl)monomethylurea derivatives, respectively. These metabolites have also been isolated from bean plants supplied with isouron. Compound S7, which was newly isolated from sugarcane, was identified as the (hydroxy-*tert*-butyl)urea derivative. The identities of S1–S4 and S7 were further

Table III. Spectroscopic Data of Isouron and Its Metabolites

	metabolites				
isouron	monomethylurea	urea	(hydroxy-*tert*-butyl)-dimethylurea	(hydroxy-*tert*-butyl)-urea	(hydroxymethyl)-urea
			EI-MS[a]		
211 (M^+)	197 (M^+)	183 (M^+)	227 (M^+)	200 ($M^+ + 1$)	213 (M^+)
167	167	140 (base peak)	197	169 (base peak)	195
154	140 (base peak)	125	183	156	183
140	125	57	154	125	167
72 (base peak)	58	44	127	55	156
44	41		72 (base peak)	44	151
			44		140 (base peak)
					125
					57
					41
			^{1}H NMR[b]		
1.28 (9 H, s, *t*-Bu)	1.35 (9 H, s, *t*-Bu)	1.34 (9 H, s, *t*-Bu)	1.32 (6 H, s, *N*-Me_2)	1.30 (6 H, s, *N*-Me_2)	
3.03 (6 H, s, *N*-Me_2)	2.94 (3 H, d, J = 6 Hz, *N*-Me)	5.82 (1 H, s, 4-H)	3.04 (6 H, s, *N*-Me_2)	3.66 (2 H, s, CH_2O)	
6.60 (1 H, s, 4-H)	5.86 (1 H, s, 4-H)	6.20 (2 H, br, $CONH_2$)	3.66 (2 H, s, CH_2O)	5.89 (1 H, s, 4-H)	
8.44 (1 H, br, NH)	7.25 (1 H, br, CONH)	8.67 (1 H, s, 3-NH)	6.75 (1 H, s, 4-H)	6.20 (2 H, br, $CONH_2$)	
	9.20 (1 H, s, 3-NH)		7.27 (1 H, s, 3-NH)	7.54 (1 H, br, 3-NH)	

[a] m/z. [b] In $CDCl_3$; δ values.

confirmed by mass and ^{1}H NMR spectrometric analyses of the purified corresponding nonradioactive compounds. The spectral data of these nonradioactive compounds were identical with those of the synthetic reference specimens summarized in Table III. A compound corresponding to the (hydroxy-*tert*-butyl)monomethylurea derivative was detected on TLC plates, but its amount was too small to be analyzed by ^{1}H NMR or mass spectrometry.

The component S5 had an R_f value identical with that of the (hydroxymethyl)urea derivative in solvent system III. The radioactive zone corresponding to the component was removed from the TLC plate and eluted from silica gels with chloroform–methanol (1:1, v/v). The isolated component, however, was resolved into two radioactive spots, which had R_f values identical with those of the (hydroxymethyl)urea and urea derivatives, respectively, in the four solvent systems I–III and V. Preliminary experiment showed that the synthetic (hydroxymethyl)urea derivative easily decomposed to urea derivative on TLC plate or during isolation procedure from silica gels. Moreover, the synthetic compound stored in methanol transformed to urea derivative and an unidentified compound. A corresponding compound to S5 on TLC was purified from the sugarcane treated with unlabeled isouron, and its mass spectrum behaved identically with that of the synthetic (hydroxymethyl)urea derivative. Therefore, S5 was identified as a (hydroxymethyl)urea derivative. A similar unstable compound, 3-(4-chlorophenyl)-1-(hydroxymethyl)urea, was previously found as a metabolite of monuron [3-(4-chlorophenyl)-1,1-dimethylurea] by the in vitro microsomal oxidase system prepared from the cotton plant (Tanaka et al., 1972).

Each of the compounds S8–S15 found in the butanol fraction was purified as a single radioactive spot on TLC in solvent system IV and individually treated with several kinds of glycosidases. All of the compounds except S8 were hydrolyzed by β-glucosidase but not by α-glucosidase (from yeast, Boehringer) or α- (from coffee beans, Boehringer) and β-galactosidase (from *Escherichia coli*, Boehringer). The radioactive hydrolytic product of S9 was not acetylated and was identical with the monomethylurea derivative in its R_f values on TLC in the four solvent systems I–III and V. Therefore, S9 was tentatively identified as the O-glucoside of the (hydroxymethyl)methylurea intermediate, which was previously isolated from bean plants.

Table IV. Changes in the Distribution of Radioactivity of Isouron and Its Metabolites in Solvent Fractions from Sugarcane Plants

	^{14}C distribn, %: h after end of treatment		
	0	48	168
isouron	93.0	57.8	22.7
monomethylurea	2.9	7.7	6.4
(hydroxymethyl)urea	nd	1.8	2.4
urea	0.3	2.6	2.0
(hydroxy-*tert*-butyl)dimethylurea	0.2	0.6	0.7
(hydroxy-*tert*-butyl)monomethylurea	nd	1.2	1.5
(hydroxy-*tert*-butyl)urea	nd	7.0	14.3
S8	nd	1.1	3.9
glucoside of (hydroxymethyl)methylurea	0.3	6.6	10.5
S10	nd	0.7	1.4
S11	nd	0.2	0.7
S12	nd	0.5	1.8
glucoside of (hydroxymethyl)urea	nd	3.7	9.1
glucoside of (hydroxy-*tert*-butyl)urea	nd	0.5	4.2
S15	nd	1.0	3.3

[a] The percentages are based on the total radioactivity found in the plants.

The R_f values of the radioactive hydrolytic product of S13 coincided with those of the (hydroxymethyl)urea derivative in the solvent systems I–III and V. Moreover, the mass and ^{1}H NMR spectra of the hydrolytic product of the corresponding nonradioactive compound were same as those of the synthetic (hydroxymethyl)urea derivative. Thus, S13 was tentatively identified as the O-glucoside of the (hydroxymethyl)urea derivative. The radioactive hydrolytic product of S14 was acetylated, and the product and its acetate were identical with the (hydroxy-*tert*-butyl)urea derivative and its acetate, respectively, on TLC plate in the solvent systems II–V. S14, therefore, was tentatively identified as the O-glucoside of the (hydroxy-*tert*-butyl)urea derivative.

Time Course Study. Changes in the distribution of radioactivities of isouron and its metabolites are shown in Table IV. Isouron was rapidly metabolized in sugarcane plants. During 4-h treatment period, 7% of isouron was metabolized. Then, isouron decreased rapidly during the next 48 h to about 58% of the total radioactivity found in the plants. At 168 h, the isouron that remained unchanged in the plants was less than 23% of the total ra-

Table V. Inhibitory Activity of Isouron and Its Metabolites on Photosynthetic O_2 Evolution in *Chlorella*

compd	I_{50},[a] M
isouron	4.0×10^{-7}
monomethylurea	9.7×10^{-7}
(hydroxymethyl)urea	6.8×10^{-5}
urea	2.1×10^{-4}
(hydroxy-*tert*-butyl)dimethylurea	1.6×10^{-4}
(hydroxy-*tert*-butyl)monomethylurea	7.6×10^{-4}
(hydroxy-*tert*-butyl)urea	$>10^{-3}$

[a] I_{50} represent the molar concentration required to reduce by 50% the photosynthetic O_2 evolution.

dioactivity. About 3% of isouron converted to the monomethylurea derivative during a 4-h treatment period. After the end of treatment, the level of the monomethylurea derivative increased rapidly for 48 h, reaching 7.7%, but slightly decreased after 168 h. The second N-demethylation to form urea derivative also occurred during the 4-h treatment period. A trace of the urea derivative was detected at 0 h, which increased to 2.6% at 48 h but slightly decreased thereafter, a similar trend to that of the monomethylurea derivative. The glucoside of the N-demethylation intermediate, (hydroxymethyl)-methylurea, was also detected at 0 h and increased steadily to 6.6% at 48 h and 10.5% at 168 h. The second N-demethylation intermediate, the (hydroxymethyl)urea derivative, was not detected at 0 h but slowly accumulated, reaching 2.4% after 168 h. The glucoside of the (hydroxymethyl)urea derivative was not detected at 0 h, appeared after 48 h, and reached 9.1% after 168 h. Although a trace of the (hydroxy-*tert*-butyl)dimethylurea but not (hydroxy-*tert*-butyl)monomethylurea was detected at 0 h, formation of these compounds proceeded very slowly until 168 h. The (hydroxy-*tert*-butyl)urea derivative, which was the most abundant metabolite at 168 h, was not detected at 0 h, but it together with its glucoside increased rapidly during the metabolic period.

Inhibition of Photosynthetic O_2 Evolution by Isouron and Its Metabolites. Inhibitory activities of isouron and its metabolites on photosynthetic O_2 evolution in *Chlorella* are shown in Table V. Isouron showed the most potent inhibitory activity and its activity was similar to that of DCMU ($I_{50} = 2.1 \times 10^{-7}$), linuron ($I_{50} = 4.2 \times 10^{-7}$), and DCPA ($I_{50} = 4 \times 10^{-7}$).

The first N-demethylation to form the monomethylurea derivative increased the I_{50} value more than twofold. Hydroxylation of the *N*-methyl group of monomethylurea derivative to form the (hydroxymethyl)urea derivative further increased the I_{50} value 2 orders of magnitude. The second N-demethylation to form the urea derivative and hydroxylation of the *tert*-butyl group significantly increased the I_{50} values: 3 orders of magnitude as compared to that of isouron. Thus, (hydroxy-*tert*-butyl)urea derivative showed almost no effect on photosynthesis.

DISCUSSION

All the results obtained in this study indicate that the metabolic pathway of isouron in sugarcane, a resistant species, is essentially the same as that in bean plants, a susceptible species, except that in sugarcane the metabolism proceeds further than in bean (Figure 4) (Ishizuka et al., 1982a).

The sequential N-demethylation to form monomethylurea and urea derivatives occurred rapidly. Formation of monomethylurea and urea derivatives was detected at the end of the 4-h treatment period, and these metabolites increased rapidly for further 48 h. The stepwise N-demethylation proceeds via the unstable (hydroxymethyl)-methylurea and (hydroxymethyl)urea derivatives that were

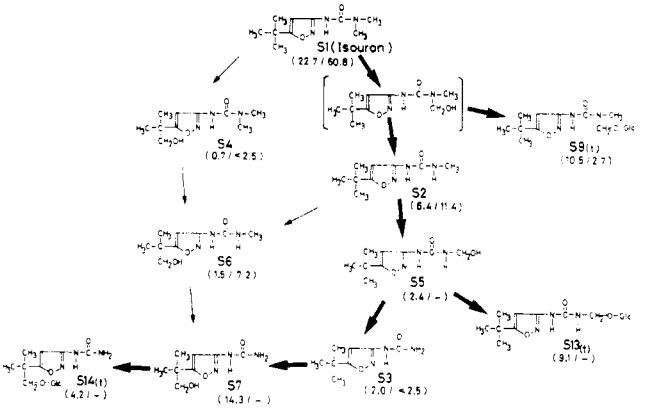

Figure 4. Presumed metabolic pathways of isouron in sugarcane seedlings. t indicates tentative identification. The compound in brackets is the postulated intermediate. Values in parentheses are the percent activities of isouron and its metabolites in sugarcane and bean plants (according to Ishizuka et al., 1982a) at 168 h after a 4-h root application of [^{14}C]isouron.

conjugated to glucose. The glucosides of (hydroxymethyl)methylurea and (hydroxymethyl)urea derivatives accounted for a large portion of accumulated metabolites. Hydroxylation of the *tert*-butyl group occurred concurrently with N-demethylation. Formation of the (hydroxy-*tert*-butyl)dimethylurea derivative was also detected at the end of treatment. But, formation of the (hydroxy-*tert*-butyl)dimethylurea and (hydroxy-*tert*-butyl)-monomethylurea derivatives proceeded very slowly until 168 h. The most abundant metabolite found after 168-h metabolism was the (hydroxy-*tert*-butyl)urea derivative, which was also glucosylated. But, the possible intermediates that must be formed by sequential N-demethylation of the (hydroxy-*tert*-butyl)dimethylurea derivative to the (hydroxy-*tert*-butyl)urea derivative, and their conjugates, were not detected in this study. Therefore, it is likely that the sequential N-demethylation of isouron to monomethylurea and urea derivatives followed by hydroxylation of the *tert*-butyl group to (hydroxy-*tert*-butyl)urea derivatives is the major route of metabolism of isouron in sugarcane plants.

Sugarcane converted isouron more rapidly than bean. The unaltered isouron contained 23% and 61% of the total radioactivity at 168 h in sugarcane and bean, respectively. Sugarcane was able to metabolize isouron to the (hydroxy-*tert*-butyl)urea derivative and to form rapidly glucose conjugates of hydroxylated intermediate metabolites, but bean was not. At 168 h after treatment the percents of the (hydroxy-*tert*-butyl)urea derivative and total glucosides (S9–S15) were about 14% and 31% of the total radiactivity found in the plants, respectively. The (hydroxy-*tert*-butyl)urea derivative was almost not active on photosynthetic O_2 evolution, and all the glucose conjugates were probably nonphytotoxic. On the other hand, in bean plants the major metabolite is the monomethylurea derivative, which was half an activity of isouron to inhibit photosynthetic O_2 evolution. Thus, at 168 h after treatment the total percent of active compounds, i.e. isouron and monomethylurea derivative, is greater in bean (72%) than in sugarcane (29%).

These differences in metabolic activity between resistant sugarcane and susceptible bean and in inhibitory activity on photosynthesis among metabolites constitute the basis for herbicidal selectivity.

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pH-Dependent Adsorption Isotherms of Glyphosate

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The relationship of system pH to glyphosate adsorption was investigated in Al^{3+}, Ca^{2+}, and Na^{+} saturations of two smectites (montmorillonite and nontronite), NaCl-washed kaolinite, hematite, and goethite. Batch equilibria techniques were used to elucidate adsorption isotherms for these minerals at four specific pH values. Glyphosate adsorption generally decreased as the system pH was increased, although there were exceptions. The exchangeable cation of the smectites influenced glyphosate adsorption capacities; Al^{3+}-saturated smectite adsorbed more glyphosate than Ca^{2+} followed by Na^{+}. Nontronite was found to adsorb more glyphosate than montmorillonite for the same saturating cation and pH. Glyphosate adsorption characteristics of kaolinite, hematite, and goethite were dependent on the pH of the clay–glyphosate system. The amount of glyphosate adsorbed was determined by the charge of the mineral surface and the ionic state of the glyphosate. The increased negative charge of glyphosate and the mineral surfaces with increasing pH resulted in decreased adsorption.

INTRODUCTION

Glyphosate is the active ingredient of the herbicide Roundup (Mullison et al., 1979). This chemical is a nonselective herbicide that is readily translocated to meristematic regions of plants. The ability to translocate and kill the growing points gives glyphosate tremendous potential in the control of such rhizome-producing weeds as johnsongrass and quackgrass (Crawford and Rogers, 1973; Claus and Behrens, 1976).

No-till production systems have employed a glyphosate solution to kill all plants in a field before planting. Planting can begin as soon as the kill has taken place with no crop damage (Peters and Dest, 1975). The lack of damage to crops planted immediately after such a treatment is apparently due to the rapid inactivation of the herbicide in the soil. Two reasons for the inactivation of soil-applied glyphosate have been postulated: (1) decomposition by soil microbes and (2) sorption of the whole molecule to soil components (Sprankle et al., 1975). Adsorption, at least initially, would seem to better account for the behavior of glyphosate where deactivation is immediate.

Adsorption has characteristically been one of the most important factors influencing the behavior of herbicides in soil systems (Hensley et al., 1978). Glyphosate adsorption has been studied on whole soils and individual minerals by bioassay or radioisotope techniques (Hance, 1976; Sprankle et al., 1975; Nomura and Hilton, 1977; Damanakis, 1976). These reports on glyphosate adsorption vary because of the range in chemical and physical properties of soils employed, the differences in dosage rates, and the contrasting analytical methods.

The purpose of this study was to quantify the adsorption of the four different ionic species of glyphosate onto several soil minerals of the clay size fraction. This would help in understanding the mechanism of glyphosate deactivation as well as allow estimation of the adsorption capacities of the minerals for glyphosate.

Table I. Surface Area, Cation-Exchange Capacity, and Zero Point of Charge for Montmorillonite, Nontronite, Kaolinite, Hematite, and Goethite

	cation-exch capacity, cmol kg^{-1}	surface area, m^2 g^{-1}	zero pt of charge: pH
montmorillonite	98	813	
nontronite	142	908	
kaolinite	16.2	37.2	3.7
hematite	0.7	11.1	7.9
goethite	6.9	50.7	8.4

EXPERIMENTAL SECTION

Clay Minerals. Clays selected for this study were Oklahoma montmorillonite, Washington nontronite, Georgia Kaolinite, and synthetic hematite and goethite. The smectites were dispersed by agitation in deionized water and then sedimented to separate the clay-sized fractions (less than 2 μm in diameter). These clays were then divided into approximately equal samples and treated

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