Thus, both the parent compound and at least one of its breakdown products can be lost by co-distillation.

The leveling off of the radioactivity in the vials after the water had been removed (see Figure 6) can be explained on the basis of adsorption of the compounds on the surface of the glass and the formation of dry water insoluble decomposition products.

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Degradation and Metabolism of Drepamon in Rice and Barnyard Grass

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The fate of $C_6H_5{}^{14}CH_2SCON\text{-}(sec\text{-}C_4H_9)_2$ ([^{14}C]Drepamon), applied in preemergence to overspreading water, was investigated in a system including soil, water, and rice plants. A product of microbiological oxidation was isolated from both water and plants and identified as $HOOC^{14}CH_2SCON\text{-}(sec\text{-}C_4H_9)_2$, whose formation was strictly related to the presence of soil. Among the metabolites of [^{14}C]Drepamon two compounds were identified by cochromatography with its chemical oxidation products as $C_6H_5{}^{14}CH_2S(O)CON\text{-}(sec\text{-}C_4H_9)_2$ and $C_6H_5{}^{14}CH_2S(O)_2CON\text{-}(sec\text{-}C_4H_9)_2$. The former showed unspecific herbicidal activity when applied to demineralized water as the only growing medium for both rice and barnyard grass. The selective action of Drepamon appeared to be unrelated to the level of this metabolite in the plant as a whole and is better explained in terms of different absorption and metabolism of the herbicide in the crop with respect to the weed.

S-Benzyl *N*,*N*-di-*sec*-butylthiolcarbamate (Drepamon) is a new herbicide developed for the selective control of barnyard grasses (*Echinochloa crusgalli* (L.) Beauv. and *Echinochloa colonum*) in rice (*Oryza sativa* L.). Although its recommended dosage in both pre- and postemergence treatments is 4 kg/ha, it appeared not to adversely affect most varieties of rice even at 15 kg/ha, under laboratory conditions (Arsura, 1972).

The present work was undertaken to investigate its fate in each of the components of the ternary system water–soil–crop, under conditions simulating a flooded paddy field. The finding that a metabolite of Drepamon had high, although unspecific, herbicidal activity prompted us to extend part of the work to a differential study in barnyard grass and rice, under various conditions, and contributed to the discovery of a new class of herbicides (Santi et al., 1974; Gozzo et al., 1975). Similar approaches and advances were at the same time independently followed by Stauffer researchers and Casida et al. (1974).

Soil degradation and plant metabolism of thiolcarbamates have received moderate attention, so far. All the works published point out a hydrolytic cleavage of the sulfur–carbamoyl bond as the main pathway together or after N-dealkylation (Kearney and Kaufman, 1969). The last reported study with *S*-(4-chlorobenzyl)-*N*,*N*-diethylthiocarbamate (Benthiocarb) did not add anything to change this picture (Ishikawa et al., 1973).

However, when the present work had already been concluded, Casida et al. anticipated evidence in favor of sulfoxidation of thiolcarbamate herbicides as an intermediate step of their metabolism both in the liver of mice (Casida et al., 1975) and in plants (Lay et al., 1975). Their attempts to show the presence of the corresponding sulfoxide in the liver of Benthiocarb-treated mice failed.

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MATERIALS AND METHODS

$C_6H_5{}^{14}CH_2SCON\text{-}(sec\text{-}C_4H_9)_2$ ([^{14}C]Drepamon) had a specific activity of 45 μCi/mg with a radiochemical purity higher than 98%. It was always applied as a 70% liquid formulation. Authentic samples of the following compounds were used to identify the degradation products of Drepamon: *N*,*N*-di-*sec*-butylcarbamoylthiolglycolic acid (compound A), obtained as described later; benzyl *N*,*N*-di-*sec*-butylcarbamoyl sulfoxide (B) and benzyl *N*,*N*-di-*sec*-butylcarbamoyl sulfone (C), prepared by oxidation of Drepamon according to a method previously described (Santi et al., 1974); dibenzyl disulfide (D), a commercial sample.

Preparation of A. Sodium hydroxide (24 g, 0.6 mol) in water (20 ml) was added to a solution of thiolglycolic acid (27.6 g, 0.3 mol) in benzene (300 ml) and the water was removed by azeotropic distillation. To the vigorously stirred boiling slurry di-*sec*-butylcarbamoyl chloride (57.5 g, 0.3 mol) was added dropwise and the mixture was kept under reflux for 4 h and then cooled and treated with water (300 ml). The aqueous layer was separated, acidified with 5 N hydrochloric acid, and extracted with dichloromethane (2 × 100 ml). The combined extracts were washed with water (300 ml), dried (Na_2SO_4), and evaporated under reduced pressure to give a yellowish oil (23.0 g). This was then dissolved in 1 N sodium hydroxide (450 ml), the resulting solution was extracted with 1-butanol (450 ml), and on evaporation of the latter a solid was obtained that was taken up with an excess of 1% hydrochloric acid. The mixture was extracted with diethyl ether (70 ml), the solvent was evaporated, the residue was dissolved in

chloroform (80 ml), and the solution was boiled with active charcoal, filtered, and evaporated under reduced pressure to give di-*sec*-butylcarbamoylthiolglycolic acid (13.0 g), a white solid: mp 48.5 °C; ν_{max} 1712 (–COOH) and 1661 cm^{-1} (>NC(=O)) (in CS_2); mass spectrometry gave a top mass peak at 247 [$C_{11}H_{21}NO_3S^+$] and a consistent fragmentation pattern (Bucci, 1971). Anal. Calcd for $C_{11}H_{21}NO_3S$: N, 5.66; S, 12.96. Found: N, 5.44; S, 12.45.

Separation of B into Diastereoisomers. Compound B, obtained by oxidation of Drepamon with 1 mol of 3-chloroperoxybenzoic acid, was resolved by thin-layer chromatography on silica gel using solvent system II (see Table I) into three components, B_1, B_2, and B_3.

Each of them was isolated in a Merck silica gel G 20 × 2.5 cm column using as the eluting system *n*-hexane + acetone (4:1). With a rate elution of 1.2 ml/min, the following fractions, each 12 ml, were collected: fractions 6 and 7 (component B_3), fractions 9 and 10 (component B_2), and fractions 13 and 14 (component B_1). After evaporating the solvent, the three components gave the following elementary analyses. Anal. Calcd for $C_{16}H_{25}NO_2S$ (mol wt 295.4): C, 65.04; H, 8.53; N, 4.74; S, 10.85. Found, B_1: C, 64.67; H, 8.61; N, 4.41; S, 10.31. Found, B_2: C, 64.35; H, 8.80; N, 4.38; S, 10.69. Found, B_3: C, 64.49; H, 8.66; N, 4.41; S, 10.73.

Infrared analysis in CS_2 gave for all three components the same spectrum: ν_{max} 1695 (C=O) and 1074, 1063, and 1050 cm^{-1} (S→O). Mass spectra showed a common top mass peak at 295. On the basis of these data the three components were deduced to be diastereoisomers of the common structure B.

Characterization of C. Compound C was obtained as a white solid, mp 73.5–74 °C (ligroin) in the same way as compound B, but using 2 mol of the peracid, and was characterized as follows: ir (KBr) ν_{max} 1685 (C=O) and 1305 and 1126 cm^{-1} (SO_2); MS *m/e* 311 (M^+). Anal. Calcd for $C_{16}H_{25}NO_3S$ (mol wt 311.4): N, 4.50; S, 10.29. Found: N, 4.32; S, 10.11.

All the bioassays with rice and barnyard grass were carried out using seed germinated on wet paper for 48 and 72 h, respectively, at 25 °C. Greenhouse and outdoor tests were carried out in vessels of 1100 and 4000 cm^2 cross section, respectively, where soil, designated as silty sand loam properly fertilized, had been laid and flooded with water at a level over the soil surface of 9 and 5 cm, respectively. Sowing was done with 32 seeds every 1000 cm^2 of surface. Then a 70% liquid formulation of [^{14}C]Drepamon was applied to the overspreading water. The level of the latter in the greenhouse tests was kept constant until the end, while in the outdoor tests it was increased up to 12.5 cm at 13 days after the application and kept at this value for a further 60 days. After that period no more water was added to the system.

The tests in the absence of soil were carried out in glass vessels of 190 cm^2 cross section. In each of them 20 seeds of rice or 30 seeds of barnyard grass, laid on a suitably drilled plate, were dipped in 1 l. of water, whose level was 2 cm above the seeds. As growing medium either deionized water or a nutrient solution was used, the latter containing the following amounts of salts (per liter): 24.6 mg of $MgSO_4 \cdot 7H_2O$, 18.6 mg of KCl, 40.1 mg of NH_4Cl, 6.8 mg of KH_2PO_4, 54.7 mg of $CaCl_2 \cdot 6H_2O$, 3.7 mg of iron citrate, and half-strength dosages of microelements according to Hoagland. The vessels, covered with glass plates, were kept in a growth room with a 15-h photoperiod, a light intensity of 5000 lx, and a daily excursion of temperature between 15 and 25 °C. The tests of herbicidal activity were carried out in a growth room operating at a light intensity of 2500 lx and a temperature between 17.5 and 31 °C.

Table I. R_f Values on Thin-Layer Plates of Authentic Reference Compounds

	Solvent system[a]			
	I	II	III	IV
Drepamon	0.44	0.88	0.84	0.88
Compound A	0.00	0.00	0.40	0.13
		0.66[b]		0.66[b]
Compound B	0.00	0.59[b]	0.76	0.59[b]
		0.52[b]		0.52[b]
Compound C	0.00	0.84	0.80	0.84
Compound D	0.67	0.84	0.77	0.84

[a] Solvent system (v/v: I = *n*-hexane + ethyl acetate (97:3), two migrations; II = *n*-hexane + acetone (2:1), two migrations; III = 1-butanol saturated with 1 N NH_4OH, one migration; IV = II, followed by one migration with chloroform + cyclohexane + acetic acid (73:20:7) for 6 cm from starting line. [b] Diastereoisomers (see later in the text).

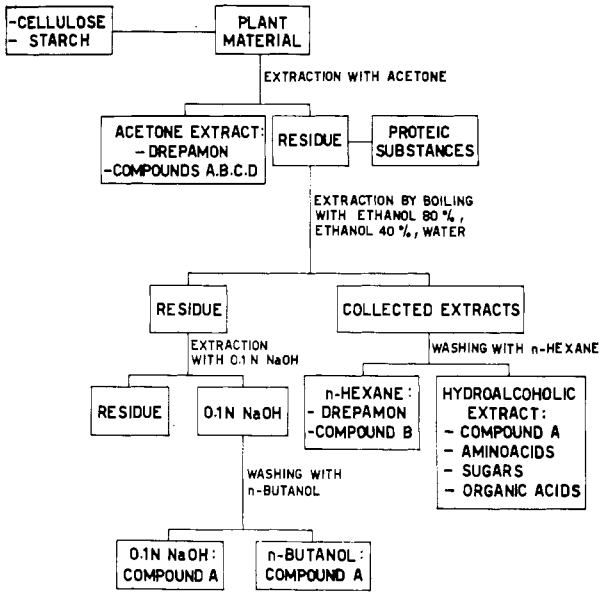

Figure 1. Purification scheme.

Analyses were performed on samples of water and soil and on single plants, at different times after the application. ^{14}C in the former was measured with a Model 3320 Packard Tri-Carb using standard channels-ratio quench correction curves. Radioactivity in the vegetables and in the soil was measured after combustion in a Model 305 Packard Sample Oxidizer by liquid scintillation counting of the liberated $^{14}CO_2$.

Drepamon and compounds A, B, C, and D were detected by thin-layer chromatography (TLC) on prelayered silica gel plates of 0.3-mm layer thickness. The solvent systems used are reported in Table I.

Radiolabeled components were revealed by autoradiography using Ferrania 3M Co. type T and quantitatively determined by counting of the separate bands of the chromatograms after their removal and suspension in a liquid scintillation counter (Instagel Packard). Drepamon and compounds A, B, C, and D were also revealed on developed chromatoplates by spraying $PdCl_2$ reagent.

The purification procedure followed to isolate individual compounds and natural substances starting from plant tissues is outlined in Figure 1. Proteic substances in the whole rice plant, except caryopsides, were isolated as follows: 2 g of plant residue, left after acetone extraction, was dispersed with 50 ml of 3 N NaOH; the mixture was

stirred for 4 h and then kept at room temperature for a further 24 h. After that time, the suspension was centrifuged at 10000*g* for 30 min. The supernatant liquid (25 ml) was added to ethanol (75 ml) and centrifuged at 10400*g* for 15 min.

From the ethanolic phase, after removal of the solvent under reduced pressure, the proteic substances were precipitated according to Pratolongo (1952) with an average yield of 42% on the basis of N in the starting material. Starting from the caryopsides, the proteic substances therefrom were isolated as follows: after acetone extraction, the residue was heated with 1:1 w/v 0.1 N NaOH, stirred for 20 min, and kept at room temperature for 24 h. Then the suspension was centrifuged at 1000*g* for 20 min and methanol was added to the supernatant phase in a ratio of 1:1.3 to precipitate starch. Centrifugation at 23500*g* was further continued for 40 min at 0 °C and proteic substances were precipitated from the supernatant solution by adding 0.2 N HCl up to pH 6.4. The yield of proteic substances was 72% on the basis of N present in the caryopsides.

Amino acids were isolated by two-dimensional TLC on plates coated with a 0.3-mm layer consisting of silica gel H and cellulose (22:8, w/w), using the following eluting systems: (1) 1-butanol + acetone + 28 Bé ammonia + H_2O (10:10:5:2); (2) 2-propanol + 99% formic acid + H_2O (20:1:5).

Detection was carried out by spraying the plates with a chromogenous reagent consisting of 0.2 g of ninhydrin, 8 ml of ethanol, 2 ml of collidine, and 10 ml of glacial acetic acid, and then by heating to 100 °C for 10 min. By percolating the hydroalcoholic extract on both the H^+ form of Dowex-50W and the OH^- form of Dowex-1 resin columns, the substances passing through them were considered to be sugars.

Citric, malic, glycolic, lactic, and succinic acids were isolated from the hydroalcoholic extract by two-dimensional TLC on silica gel G, 0.3 mm thick, with the following eluting systems: (1) chloroform + cyclohexane + acetic acid (73:20:7); (2) 2-propanol + 1 N ammonia (70:30). Detection was carried out with a reagent consisting of 60 ml of 1-butanol, 18 ml of distilled water, 18 ml of ethanol, and 2 g of aniline. Finally, starch and cellulose were isolated by directly working out the plant material according to methods already described in the literature (AOAC, 1965; Villavecchia, 1943).

As to the soil, TLC was carried out on extracts obtained by treating it with methanol first and then with methanol added to 2.5% HCl in the ratio soil:solvent = 1:10. Partial esterification of the carboxylic group of compound A takes place on treating the soil with acidic methanol. The reported figures for this product include its methyl ester, the R_f values of the latter being 0.10, 0.80, and 0.78 according to the solvent systems I, II, and III, respectively.

RESULTS

Plants–Soil–Water System. Experiments carried out in the greenhouse under conditions simulating a paddy field show that Drepamon is easily absorbed already in the first stages of germination by both rice and barnyard grass. This is shown in Figure 2, where the weight values of total radioactivity, expressed as Drepamon equivalents and of Drepamon (active ingredient) itself, detected in the plant extracts, are reported vs. time, starting from the date of application.

At a dosage of 15 kg/ha of active ingredient applied to the water of the rice vessels, the amount of total ^{14}C in these plants increases regularly with time, together with the normal development of the crop, and reaches a

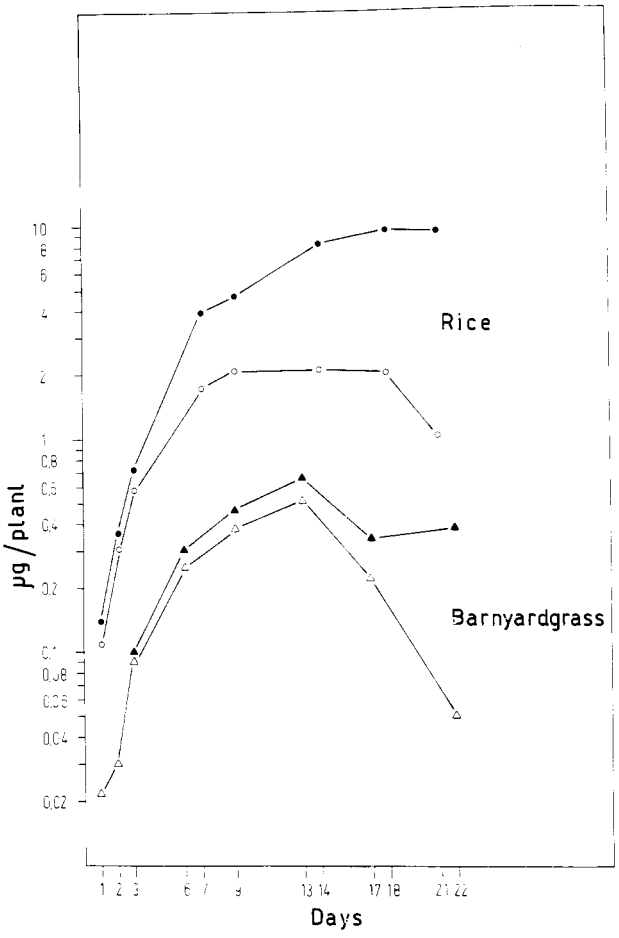

Figure 2. Greenhouse treatments, simulating a flooded paddy field. Amounts of total ^{14}C (●) and [^{14}C]Drepamon (○) in rice (applied dosage of [^{14}C]Drepamon = 15 kg/ha) and corresponding values (▲ and △) in barnyard grass (applied dosage of [^{14}C]Drepamon = 1 kg/ha).

maximum value of ca. 10 µg/plant 18 days after the application. The curve relative to the single active ingredient in the rice plants begins to gradually diverge from that of total radioactivity on the third day after the application. The divergence becomes very pronounced on the seventh day, when the amount of active ingredient in the plant reaches the maximum level of ca. 2 µg/plant. The difference between the two above-mentioned curves gives a measure of the degradation of Drepamon resulting from the combined effect of the microbiological attack in the soil and the metabolism inside the plant, the contribution by the latter being the most important one.

Figure 2 also shows the trends of the same two quantities detected in the barnyard grass plants following the same methods, but applying in this case a dosage of only 1 kg/ha of active ingredient. Although this is 15 times lower than that applied to the rice crop, where no inhibitory effect was noticed, a clear inhibition of the growth of the weed was evident from the seventh day after the application. Unlike that which occurs in the rice, the amounts of total ^{14}C and active ingredient in barnyard grass are nearly coincident up to the 13th day after the application, indicating a comparatively modest metabolic activity up to this date. A rapid fall of the level of Drepamon in barnyard grass is noticed after this time.

Taking into account the different weight development of the two species of plants a more significant comparison is obtained when the levels of total ^{14}C and active ingredient are referred to the unit weight of the plants. Figure 3 shows that, expressed in such a way, the levels

Table II. Outdoor Test Simulating a Paddy Field, Treated with 10 kg/ha of $C_6H_5{}^{14}CH_2SCON$-(*sec*-$C_4H_9)_2$ (Drepamon); Concentrations of ^{14}C-Containing Products (All Expressed as Drepamon Equivalents, ppm, w/w)

Time from application, days	Total ^{14}C	Drepamon	Compounds A	Compounds B	Compounds C	Cellulose	Starch	Proteins
			(a) In Rice Plants					
2	20.7	18.8	0.12	0.51	<0.005			
7	53.1	45.9	0.76	1.72	<0.005			
13	92.5	52.2	1.34	3.62	0.03			
27	53.6	10.7	0.58	1.69	0.35			
50	10.1	0.2	0.48	0.10	<0.005	1.42	0.21	
99	1.1	0.04	0.13	0.007	<0.005	0.12	0.04	0.27
139	1.5	0.14	0.16	<0.005	<0.005	0.13	0.03	0.27
167	1.4	0.06	0.14	<0.005	<0.005	0.13	0.02	0.20
			(b) In Overspreading Water					
2	8.9	6.3	2.1	0.12	0.06			
7	4.1	2.9	0.7	0.16	0.02			
13	2.8	1.0	1.3	0.08	<0.005			
27	5.8[a]	0.12	4.6	0.25	<0.005			
50	4.4[a]	<0.005	3.4	<0.005	<0.005			
			(c) In Soil					
2	1.5	1.4	0.02					
7	2.1	1.9	0.04					
13	3.1	2.4	0.16					
27	0.75	0.28	0.16					
50	0.33	0.06	0.15					
99	0.59	0.03	0.37					
167	0.66	0.06	0.40					

[a] The experimental values have been multiplied by a factor of 2.5 to account for the increase of the water level (see Materials and Methods).

of total ^{14}C in rice and barnyard grass are very similar at least up to 13 days from the application. In other words, the barnyard grass plants absorb an overall amount of ^{14}C-containing substances, by weight unit, comparable with that absorbed by the rice plants, although they have been treated with a dosage 15 times lower than the latter ones.

A more thorough investigation on the fate of Drepamon and products therefrom in the rice crop was carried out in an outdoor test under conditions simulating a paddy field. The dosage of Drepamon applied to the overspreading water was 10 kg/ha, about 2.5 times larger than that suggested for the total inhibition of barnyard grass. At different times from the application, the distribution of ^{14}C-containing substances was determined in the rice plants, in the soil, and in the overspreading water.

Among various ^{14}C-containing products the presence of the following compounds was observed by cochromatography with authentic samples both in the plant extracts and in the overspreading water: di-*sec*-butylcarbamoylthiolglycolic acid (compound A), using each of the two solvent systems III and IV (see Table I), and benzyl di-*sec*-butylcarbamoyl sulfoxide (compound B) and benzyl di-*sec*-butylcarbamoyl sulfone (compound C), using each of the two solvent systems II and III (see Table I).

To obtain clear evidence about the origin and the identity of compound A, it was isolated by extraction with 1-butanol from the water overspreading the soil treated with Drepamon in the absence of plants. It was purified according to the same procedure described under Materials and Methods for the sample obtained by synthesis. Its structure was then unequivocally allocated on the basis of characterization elements identical with those reported for the authentic sample and of the identity of their ir spectra. Compounds B and C had been prepared by oxidation of Drepamon with 3-chloroperoxybenzoic acid (Santi et al., 1974) as possible models of its metabolic oxidation.

The presence of the three above-mentioned compounds and of dibenzyl disulfide (compound D) in all the extracts analyzed in the present work was then checked by cochromatography with authentic samples under various conditions. Compound B, when obtained by synthesis, was resolved by TLC into three components, B_1, B_2, and B_3, that were mixtures of diastereoisomers, in the proportion of 2:5:3. In the plant extracts one of these components, B_2, appeared to predominate. Dibenzyl disulfide, another possible product of metabolism of Drepamon, was present only in traces.

Table III. Distribution of Products in the Rice Plant, Caryopsides Free, from Outdoor Test (Conditions as in Table II); Concentrations of ^{14}C-Containing Products (ppm of Drepamon Equivalent, w/w) in the Roots (R) and Aerial Part (AP)

Time from application, days	Drepamon R	Drepamon AP	Compounds A R	Compounds A AP	Compounds B R	Compounds B AP
50	0.81	0.07	0.88	0.38	0.53	0.22
99	0.17	0.004	0.40	0.05	0.02	0.003
139	0.50	0.005	0.43	0.06	<0.005	<0.005
167	0.27	0.004	0.48	0.05	<0.005	<0.005

Starting from extracts of plants collected after 50 days from the application, the content of ^{14}C was determined in the following natural substances: cellulose, starch, and proteins. No trace of ^{14}C was present in amino acids, sugars, and citric, malic, glycolic, lactic, and succinic acids.

Distribution of ^{14}C into compounds and natural constituents of extracts from rice plants under these conditions is summarized in Table II, part a. As far as the distribution of single products in the plants is concerned, analysis of the extracts showed that Drepamon was mostly localized in the roots. Compounds A and B were more evenly distributed between roots and the emergent part of the plant after 50 days from the application, the ratio being in favor of the roots at longer times (Table III).

Analysis of the water overspreading the soil showed the presence of compounds A, B, and C in addition to Drepamon, whose concentration was falling under the limits of analytical detection after 50 days from the application.

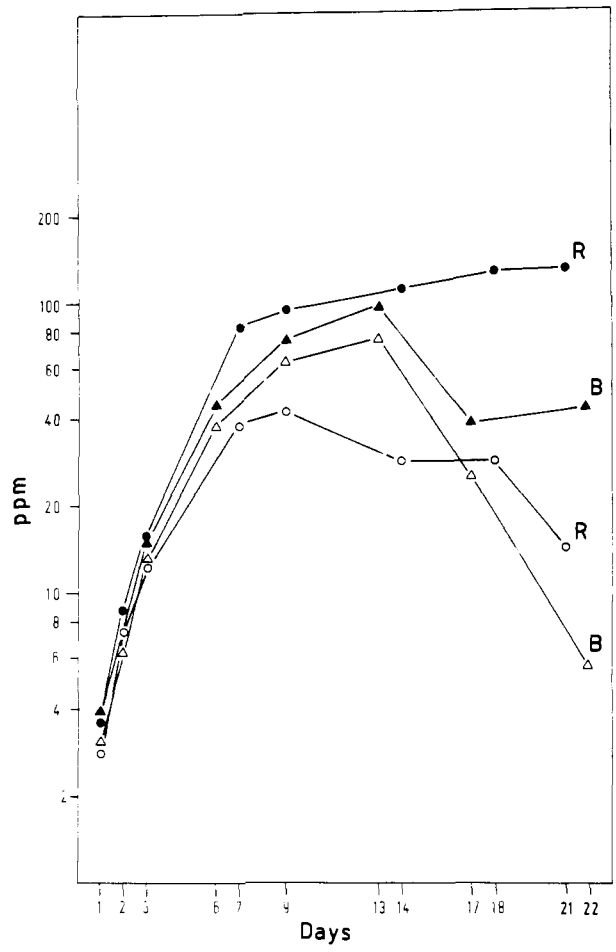

Figure 3. Greenhouse treatments. Conditions are as indicated in Figure 2. Concentrations of total ^{14}C (●) and [^{14}C]Drepamon (○) in rice (R) and corresponding values (▲ and △) in barnyard grass (B).

Compound A was the main product.

TLC analysis showed the presence of the three diastereoisomers B_1, B_2, and B_3 in the same ratio as that of the sulfoxide obtained by synthesis. The quantitative determination of compound B was expressed as the sum of the three corresponding chromatographic plots (Table II, part b). Analysis of the soil showed the presence of compound A as the only product identified. This was again the principal derivative of Drepamon (Table II, part c).

At the harvest (167 days after treatment), the content of ^{14}C present in the caryopsides and expressed as starting material was 1.1 ppm, evenly distributed in the seed and shared among the following substances: starch, 71.7%; proteins, 16.8%; cellulose, 2.6%; unidentified compounds, 8.9%. Within the limits of analytical sensitivity (0.005 ppm) no trace of starting material and compounds A, B, and C was detected.

Plants–Water System. To eliminate the contribution of the microflora to the degradation of Drepamon, the fate of the latter was studied in the absence of soil. An initial comparative analysis was carried out in the extracts of plants of rice and barnyard grass, grown in two series of vessels containing distilled water, to which Drepamon had been added at the following rates: 2 ppm of active ingredient in the rice tests; 1 ppm of active ingredient in the barnyard grass tests. The duration of the tests was limited to the first 10 days after the application. The amounts of total ^{14}C and active ingredient in the plant extracts (both referred to the single plants) are reported vs. time in Figure 4. The trends confirm the difference in the

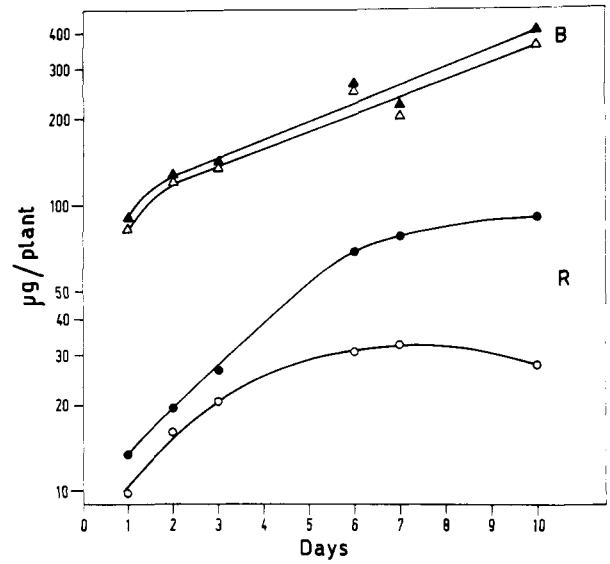

Figure 4. Laboratory treatments in demineralized water. Amounts of total ^{14}C (●) and [^{14}C]Drepamon (○) in rice (R) (applied dosage of [^{14}C]Drepamon = 2 ppm) and corresponding values (▲ and △) in barnyard grass (B) (applied dosage of [^{14}C]Drepamon = 1 ppm).

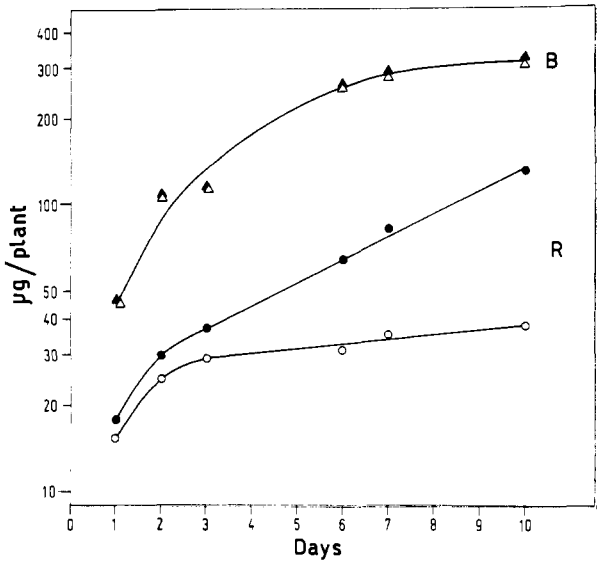

Figure 5. Laboratory treatments in nutrient solution. Amounts of total ^{14}C (●) and [^{14}C]Drepamon (○) in rice (R) and corresponding values (▲ and △) in barnyard grass (B); applied dosage, 2 ppm in both cases.

metabolic intensity between rice and barnyard grass. While the latter absorbs a considerably higher amount of active ingredient, this is metabolized very little, by comparison with what occurs in rice. Although the trends in the latter are similar to those observed in the presence of soil, a clear inhibition of growth of the rice plants comparable to that of barnyard grass was observed under these conditions after 6 days from the application. The tests were repeated by replacing the distilled water with a nutrient solution as the growing medium. The dosage of herbicide applied to the solution was for both the species 2 ppm of active ingredient. Under these conditions the selectivity was completely restored. No inhibition was noticed in rice in spite of the similarity of the trends of ^{14}C and active ingredient (Figure 5) with those obtained in the absence of salts.

To verify the selectivity of Drepamon toward rice, its content in the nutrient solution was increased up to 10

Table IV. Overall Concentration (ppm) of ^{14}C, Drepamon, and Its Metabolites in Plants of Rice and Barnyard Grass Grown in Water

Dose of [^{14}C]-Drepamon applied (referred to H_2O)	Time from application, days	Plant	ppm referred to the wt of the plants				
					Metabolites		
			Total ^{14}C	Drepamon	B	C	D
2 ppm	1	Rice	17.8	15.4	0.6		0.02
	2		29.8	24.9	1.2	0.06	0.05
	3		37.2	29.3	1.5	0.22	
	6		64.3	31.1	3.2	0.22	0.08
	7		82.2	35.2	4.2	0.36	
	10		130.4	37.8	6.6	0.56	0.07
1 ppm	1	Barnyard grass	46.8	45.6	0.2	0.2	
	2		109.0	105.2	0.7	1.4	
	3		117.1	113.3	0.6	1.3	
	6		265.7	252.4	1.3	1.9	
	7		293.0	276.5	1.6	3.2	
	10		325.6	302.4	5.4	1.3	0.55

Table V. Overall Concentrations (ppm) of ^{14}C, Drepamon, and Its Metabolites in Plants of Rice and Barnyard Grass Grown in a Nutrient Solution

Dose of [^{14}C]-Drepamon applied (referred to solution)	Time from application, days	Plant	ppm referred to the wt of the plants				
					Metabolites		
			Total ^{14}C	Drepamon	B	C	D
2 ppm	1	Rice	13.5	9.9	0.4	0.04	
	2		19.5	16.2	0.7	0.06	
	3		26.6	20.7	1.2	0.10	
	6		68.6	30.8	3.6	0.32	
	7		77.3	32.7	4.9	0.44	
	10		90.4	27.5	6.8	0.40	
2 ppm	1	Barnyard grass	90.5	83.3	1.0	0.6	
	2		129.5	122.2	1.5	0.4	0.35
	3		143.4	136.8	1.1	0.2	0.36
	6		267.2	250.2	1.5	1.2	
	7		224.5	202.0	5.1	1.7	
	10		408.4	360.0	6.4	2.2	

ppm without noticing any phytotoxicity. On the contrary, when distilled water was used as growth medium the threshold of phytotoxicity for rice was between 0.5 and 1 ppm of active ingredient. The corresponding thresholds of phototoxicity toward barnyard grass were: 0.3 ppm in the nutrient solution and 0.15 ppm in distilled water.

Some of the labeled metabolites detected in both species of plants were identified by cochromatography with the previously mentioned products. Unlike the tests in the presence of soil, no trace of compound A was present in the extracts of plants and in the water. Compound B was again resolved by TLC into the three diastereoisomers, the one with the highest R_f predominating in the rice and in the corresponding water. A more balanced distribution of these isomers was observed in barnyard grass. Their sum was, however, roughly similar in both the crop and the weed.

Compound C was present in the rice at a lower concentration than in barnyard grass. Measurable amounts of dibenzyl disulfide (compound D) were detected in some extracts of barnyard grass and more frequently but at lower concentrations in the rice grown in distilled water. Apart from this, no significant difference in the levels of the identified metabolites was noticed by replacing water with nutrient solution (Tables IV and V).

Tests of phytotoxicity were carried out with compounds B and C using distilled water as growing medium. The diastereoisomers B_1, B_2, and B_3, obtained by synthesis and isolated by TLC, were found to be more active on barnyard grass than Drepamon itself, although unlike the latter, they were not selective toward rice (Arsura, 1972). The threshold of inhibition of each of them was around 0.1 ppm for both the plants. Under the same conditions, no phytotoxicity was revealed by compound C up to a concentration of 10 ppm.

DISCUSSION

The results of the present work show that Drepamon is an easily degradable herbicide. Under the normal conditions of treatment, the amount that is not taken up by plants apparently undergoes an extensive degradation of the benzene ring to give mainly the biologically inert di-*sec*-butylcarbamoylthiolglycolic acid (A). That this is the result of an attack of the herbicide by soil microorganisms can be deduced by the absence of A in both the water and the plants grown in the absence of soil, together with the chemically unusual type of degradation. The amount of Drepamon taken up by the rice crop also undergoes an extensive metabolism. As a result, at the harvest, 167 days after the treatment, no trace of the starting material and identified metabolites was detected in the caryopsides.

The comparative analysis of the extracts from the barnyard grass and rice plants shows two significant facts. (1) Rates of $C_6H_5{}^{14}CH_2SCON$-(*sec*-$C_4H_9)_2$ being equal, barnyard grass takes up, by weight unit, a much higher amount of labeled substance than rice. This occurs already in the first stages of the vegetative development, even though this by itself results in the rice plants being heavier. (2) Metabolic activity in rice is by far more intense than in barnyard grass. This is particularly evident from the tests carried out in water as culture medium, where the interference of the soil microflora was avoided.

As an effect of the combination of these two facts, the overall concentration of Drepamon in barnyard grass is an order of magnitude higher than in rice. If Drepamon as

such is biochemically the true herbicidal agent, this observation is entirely consistent with its selectivity toward rice. When the latter is calculated as the ratio between the thresholds of concentration in water at which the growth of the two types of plants is inhibited a value exceeding 30 is obtained in nutrient solution and a value around 5 in distilled water. This large difference can be almost entirely ascribed to the interaction between the active ingredient and the rice plants, whose effect is dramatically dependent on the presence of salts in the culture medium.

The results of the present work do not allow location of the biochemical step responsible for this difference. The quantitative analysis of metabolites was mainly restricted to the products of sulfur oxidation of the active ingredient, which had been synthesized in the laboratory as hypothetical metabolites. Their effective presence in both types of plants demands a special comment in view of recent work that showed the carbamoyl sulfoxides to be a new class of herbicides (Santi et al., 1974; Gozzo et al., 1975; Casida et al., 1974). Actually, the most interesting fact that came to light during this work was the biological activity of benzyl *N,N*-di-*sec*-butylcarbamoyl sulfoxide (B). When this was tested on a number of weeds, it was shown to inhibit the growth of barnyard grass as well as other grasses at a lower dosage of Drepamon, although unlike the latter, it was not selective toward rice (Arsura, 1972). Casida et al. (1975) recently brought evidence in favor of the oxidation of a number of thiolcarbamates to carbamoyl sulfoxides by mixed function oxidases of the mouse liver, this being a first stage of detoxification, followed by attack by glutathione transferase. On the other hand, in the plants the sulfur monooxidation of thiolcarbamates might well represent a stage of increased toxicity, in accordance with the most recent findings of the same authors (Lay et al., 1975).

In the case under consideration, the presence of two *sec*-butyl groups in the molecule of B allows the existence of diastereoisomers, whose proportion in the rice plants is different from that in barnyard grass and more similar to the synthetic mixture. The fact that in the absence of soil and salts the same proportions found in the two species of plants are present in the water where they were grown indicates that a partial transfer of these components occurs from the plants to the water through the roots. Since no difference of biological activity was noticed among the three diastereoisomers obtained by synthesis, it seems convenient to consider the metabolite B as a sum of them. B shows a phytotoxicity toward rice remarkably higher than that of the parent compound (Drepamon) in salt-free water, while the increase of phytotoxicity toward barnyard grass is negligible. As a consequence, B is entirely devoid of selectivity. Since its overall concentrations in rice and barnyard grass were found to be roughly equivalent and did not undergo a substantial change on replacing distilled water with nutrient solution as culture medium, it seems improbable that B might play an important role in the herbicidal action of Drepamon.

Such a working hypothesis might not be sustained if B were found to accumulate and act in different sites according to the type of plant considered. More work is needed to establish this point and the biochemical mode of action.

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