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Fate of a Herbicide ^{14}C-Alloxydim-sodium in Sugar Beet

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A new herbicide, alloxydim-sodium(ADS)[sodium salt of 2-(1-allyloxyamino)-butylidene-5,5-dimethyl-4-methoxycarbonylcyclohexane-1,3-dione] was applied to the leaves of sugar beet (*Beta vulgaris*). The total recovery of the radioactivity of ^{14}C-ADS, labeled at C-1 position of the butylidene group, gradually decreased with half-life of 11 days. The translocation of the radioactivity to other parts of the plant was little and the residues calculated as apparent ADS in roots were 0.16, 0.0096 and 0.0020 ppm at 1.5, 4 and 6 months after the treatment, respectively. The main metabolite in the treated leaves was a deallyloxylated compound (CM-I) and minor ones were two oxo-tetrahydrobenzoxazole derivatives (CM-II, CM-III). The amounts of these metabolites reached the maximum around 10 days with 18.0% (CM-I), 2.6% (CM-II) and 2.5% (CM-III) of the applied radioactivity. This type of transformation of ADS also occurred under non-biological conditions, and the mechanism is discussed.

INTRODUCTION

Alloxydim-sodium (ADS) [sodium salt of 2-(1-allyloxyamino)-butylidene-5,5-dimethyl-4-methoxycarbonylcyclohexane-1,3-dione] is a novel selective herbicide[1] which controls graminaceous weeds without phytotoxicity towards dicotyledonous plants such as sugar beet and soybean.[2,3] In sugar beet, ADS is used as a postemergence herbicide to control annual grasses. Part of the results on the plant metabolism of this chemical was reported at the Annual Meeting of this Society.[4] The present study deals with the fate of ADS on subar beet in connection with its chemical transformation.

MATERIALS AND METHODS

1. *Spectroscopy*

Infrared spectra (IR) were recorded on a Jasco IR spectrophotometer IR-G®. Samples were analyzed as thin film on a NaCl crystal for liquid and in a KBr disc for solid. Ultraviolet spectra (UV) were determined on a Shimadzu UV spectrophotometer MPS-50L® as methanol or 0.1 N alkaline solution. Nuclear magnetic resonance spectra (NMR) were determined in $CDCl_3$, except for ADS (D_2O) and CM-VII (CD_3OD), on a Jeol NMR spectrometer JNM-MH-100® at 60 MHz. Mass spectra (MS) were obtained with a Hitachi mass spectrometer RMU-7MG® at 3.5×10^{-7} torr, 120°C and 20 eV.

2. *Chemicals*

ADS [7-^{14}C] labeled at C-1 position of the butylidene group was synthesized by the following reactions starting from sodium butyrate [1-^{14}C]. Sodium butyrate [1-^{14}C] was treated with thionyl chloride. 4-Methoxycarbonyldimedone was reacted with the ^{14}C-acid chloride in the presence of a $ZnCl_2$ catalyst to form 2-butyryl-4-methoxycarbonyldimedone [butyryl-1-^{14}C]. The latter compound was transformed to ^{14}C-alloxydim by allyloxyamination, and then to ^{14}C-ADS with sodium hydroxide. The total radiochemical yield was 12.9% from sodium butyrate [1-^{14}C]. The specific radioactivity was 2.52 μCi/mg or 0.871 mCi/mmol. The radiochemical purity of the

compound was 99% when checked by thin layer chromatography (*tlc*) using a silica gel plate (Eastman Chromagram sheet 6060®) and developing solvent of *n*–hexane:acetone (7:3).

Nonlabeled ADS and sodium-free ADS, alloxydim, were prepared by the same method as the labeling sythesis.

ADS. mp 185.5°C (decomposed). IR, ν 1740 (CO), 3460 cm^{-1} (NH); UV, $\lambda_{max}^{0.1N\ NaOH}$ 287 nm; NMR, δ from DSS 0.8 (3H, t), 1.0 (3H, s), 1.1 (3H, s), 1.3 (2H, m), 2.0 (1H, d), 2.3 (3H, m), 3.0 (1H, s), 3.5 (3H, s), 4.3 (2H, d), 5.0 (2H, m), 5.7 ppm (1H, m).

Alloxydim. $n_D^{24.5}$ 1.5063. IR, ν 1655 and 1735 cm^{-1} (CO); UV, $\lambda_{max}^{0.1N\ NaOH}$ 287 nm; MS, *m/e* 323 (M^+), 267, 266, 237; NMR, δ from TMS 1.0 (3H, t), 1.1 (3H, s), 1.6 (2H, m), 2.3 (1H, d), 2.9 (3H, m), 3.3 (1H, s), 3.7 (3H, s), 4.3 (2H, d), 5.4 (2H, m), 6.0 ppm (1H, s).

2 – (1 – Aminobutylidene) – 4 – methoxycarbonyl – 5,5 – dimethylcyclohexane – 1,3 – dione (CM–I) was obtained by amination of 2–butyryl – 1 – hydroxy – 4 – methoxycarbonyl – 5,5 – dimethyl–1–cyclohexen–3–one (CM–VI) with ammonia. $n_D^{23.5}$ 1.5406. IR, ν 1735 (CO), 3280 cm^{-1} (NH); UV, λ_{max}^{MeOH} 253, 290 nm; MS, *m/e* 267 (M^+), 236, 220, 208; NMR, δ 1.00 (3H, t), 1.12 (6H, s), 1.64 (2H, m), 2.32 (1H, d), 2.72 (1H, d), 2.92 (2H, t), 3.32 (1H, s), 3.72 (3H, s), 7.28 ppm (1H, m).

6,6–Dimethyl–5–methoxycarbonyl–2–propyl–4–oxo–4,5,6,7–tetrahydrobenzoxazole (CM–II) and 6,6–dimethyl–7–methoxycarbonyl–2–propyl – 4 – oxo – 4,5,6,7 – tetrahydrobenzoxazole (CM–III) were prepared as a mixture by refluxing alloxydim (sodium-free ADS) in ethanol for 15 hr. The distilled mixture (bp 165–167°C/0.4–0.5 mmHg) was treated with hydroxylamine in ethanol to give selectively the oxime of CM–III because of steric hindrance. The reaction mixture was dissolved in ether and then cooled at —5°C. The resulting precipitate was filtered off. The ethereal solution was washed several times with an alkaline aqueous solution to extract major parts of the remaining oxime. After evaporating the ether, the residual oil was charged on a silica gel column and CM–II was eluted with benzene:ethyl acetate (9:1). $n_D^{23.5}$ 1.5035. IR, ν 1690 and 1735 cm^{-1} (CO); UV, λ_{max}^{MeOH} 253 nm; MS, *m/e* 265, 250, 234, 233, 218, 206, 205; NMR, δ 1.00 (3H, t), 1.20 (3H, s), 1.24 (3H, s), 1.84 (2H, m), 2.80 (2H, t), 2.93 (2H, dd), 3.32 (1H, s), 3.72 ppm (1H, s). On the other hand, the CM–III oxime was dissolved in ethanol and treated with a formaldehyde solution and 1N hydrochloric acid at 50°C for 3 hr to get CM–III. Mp 82–83°C. IR, ν 1690 and 1735 cm^{-1} (CO); UV, λ_{max}^{MeOH} 248 nm; MS, *m/e* 265 (M^+), 250, 233, 218, 205; NMR, δ 1.00 (3H, t), 1.20 (3H, s), 1.24 (3H, s), 1.84 (2H, m), 2.60 (2H, dd), 2.76 (2H, t), 3.72 (1H, s), 3.78 ppm (3H, s). An alternative synthesis of CM–II. 6,6–Dimethyl–4–oxo–2– propyl–4,5,6,7–tetrahydrobenzoxazole in benzene was refluxed with 6 equivalents of sodium hydride and dimethyl carbonate for 18 hr. The filtrate of the reaction mixture was concentrated. The product was purified by column chromatography, and was identical with CM–II obtained by the previous method, on IR, UV and NMR spectra.

6,6–Dimethyl–5–methoxycarbonyl–3–propyl–4–oxo–4,5,6,7–tetrahydrobenzoisoxazole (CM–IV) and 6,6–dimethyl–7–methoxycarbonyl–3–propyl–4–oxo–4,5,6,7–tetrahydrobenzoisoxazole (CM–V) were also obtained as a mixture after the reaction of CM–VI and hydroxylamine in ethanol at room temperature. From a ligroin solution of the mixture, CM–IV was obtained by recrystallization. Mp 51–53°C. IR, ν 1680 and 1740 cm^{-1} (CO); UV, λ_{max}^{MeOH} 238 nm; MS, *m/e* 265 (M^+), 250, 224, 218, 206, 192; NMR, δ 0.96 (3H, t), 1.20 (3H, s), 1.26 (3H, s), 1.72 (2H, m), 2.80 (2H, t), 2.88 (1H, d), 3.18 (1H, d), 3.36 (1H, s), 3.72 ppm (3H, s). CM–V was separated from the mother liquid following the same method as CM–III. $n_D^{21.5}$ 1.4931. IR, ν 1680 and 1730 cm^{-1} (CO); UV, λ_{max}^{MeOH} 225 nm; MS, *m/e* 265 (M^+), 250, 224, 206, 192; NMR, ν 1.00 (3H, t), 1.20 (6H, s), 1.65 (2H, m), 2.14 (1H, d), 2.82 (1H, d), 2.84 (2H, t), 3.76 ppm (4H, s).

2–Butyryl–1–hydroxy–4–methoxycarbonyl–5,5–dimethyl–1–cyclohexen–3–one (CM–VI) was prepared by the reaction mentioned in the labeling synthesis. $n_D^{23.5}$ 1.5048. IR, ν 1665 and 1735 cm^{-1} (CO); UV, λ_{max}^{MeOH} 237 and 275 nm; MS, *m/e* 268 (M^+), 253, 221, 193; NMR, δ 0.94 (3H, t), 1.12 (6H, s), 1.65 (2H, m), 2.42 (1H, d), 2.92 (1H, d), 2.96 (2H, t), 3.30 (1H, s), 3.72 (3H, s), 18.20 ppm (1H, s).

2 – (1 – Allyloxyamino) butylidene – 5, 5 – di-

methylcyclohexane–1,3–dione (CM–VII) was obtained by allyloxyamination of demethoxycarbonylated CM–VI. $n_D^{21.0}$ 1.5089. IR, ν 1658 cm^{-1} (CO); UV, λ_{max}^{MeOH} 283 nm; MS, *m/e* 265 (M^+), 208, 192, 179; NMR, δ ppm 0.92 (3H, t), 1.08 (6H, s), 1.48 (2H, m), 2.36 (4H, s), 2.84 (2H, t), 4.56 (2H, t), 5.3 (2H, m), 6.0 ppm (1H, m).

4–Methoxycarbonyl–5,5–dimethyl–2–propionamidocyclohexane–1,3–dione (CM–VIII) was obtained by catalytic reduction of the azo compound, in butyric anhydride, which was formed from 4–methoxycarbonyldimedone and tosylsulfonylazide. $n_D^{24.5}$ 1.4290. IR, ν 3250 (NH), 1735 (CO), 1600 cm^{-1} (CONH); UV, λ_{max}^{MeOH} 286 nm; MS, *m/e* 283 (M^+), 224, 212, 181; NMR, δ 1.00 (3H, t), 1.12 (6H, s), 1.70 (2H, m), 2.40 (3H, m), 2.80 (1H, d), 3.28 (1H, d), 3.80 (3H, d), 8.2 (1H, m), 13.5 ppm (1H, m).

3. *Plant Treatment*

The sugar beet plants (*Beta vulgaris* L., POLYRAVE var.) were grown in pot culture in a greenhouse (15–28°C) after the seeds were sowed on Dec. 27th, 1975. ^{14}C–ADS (7.5 mg) and a succinate type surfactant (2.5 mg) were dissolved in 10 ml of water.* This solution (100 μl, 4.20×10^5 dpm) was dotted with a microsyringe as uniformly as possible on the upper surface of the first and second true leaves (*ca.* 10 cm^2) at early third leaf stage of the plant. The treated plants were cultured outdoors in the daytime (sunny and cloudy; 38 days) and indoors at night time and on 5 rainy days during the experiment from Jan. 26th to Mar. 8th, 1976. The plants were transplanted to a larger pot (ϕ, 18 cm) and kept in the greenhouse (22–33°C) for 6 months. The weight of the root was 0.18, 1.17, 6.5, 106 and 340 g at 7, 28, 42, 120 and 180 days, respectively.

4. *Extraction and Analysis of ^{14}C-compounds*

The plants were harvested at designated intervals and divided into the treated leaves, other leaves, and roots, and then the fresh weight of each part was recorded. The treated leaves were first washed with 6 ml of water, then with 6 ml of methanol, and then homogenized with 50% methanol in a glass mortar. The aqueous methanol extract was separated from fibrous residue by centrifugation (4,000 rpm, 5 min). The fibrous residue was extracted with 10 ml of methanol, and the extract was added to the aqueous methanol extract. The untreated leaves and the roots were separately homogenized with 50% methanol, and the extracts were obtained in the same manner. All the leaf washings and the extracts were radioassayed by a liquid scintillation spectrometer (Packard Tri-Carb 3320®) with external quenching correction. The radioactivity in fibrous residue was determined by the same method after conversion to $^{14}CO_2$ by a sample oxidizer (Packard Tri-Carb 306®).

The water washing of the treated leaves was extracted twice with 8 ml of *n*–hexane and then twice with 8 ml of dichloromethane at pH 1. The aqueous layer was extracted twice with 8 ml of dichloromethane at pH 7, then twice with 8 ml of ethyl acetate at pH 7 and again at pH 1. These fractions and the methanol washing of the treated leaves were condensed under a reduced pressure, respectively, and subjected to *tlc* analysis. *Tlc* analysis was carried out in two solvent systems using silica gel plates. The Merck Art. 5554® plate was developed with benzene : ethyl

Table 1 *Rf* values of alloxydim and authentic standards.

Name	*Rf* values[a]	
	A	B
Alloxydim	0.43	0.50
CM–I	0.11	0.36
CM–II	0.24	0.49
CM–III	0.16	0.48
CM–IV	0.55	0.66
CM–V	0.55	0.66
CM–VI	0.44	0.28
CM–VII	0.54	0.67
CM–VIII	0.13	0.10

[a] *tlc* systems
A: Merck Art 5554 was developed with benzene–ethyl acetate (4 : 1), B: Eastman No.6060 was developed with *n*–hexane–acetone (7 : 3)

* The formulation of ADS in the market is a 75% soluble powder, which is sprayed in the field as a 1,000-fold aqueous solution.

acetate (4 : 1) and the Eastman No. 6060® sheet was developed with *n*–hexane: acetone (7 : 3). The *Rf* values of authentic standards are shown in Table 1. *Tlc* autoradiogram was prepared with a medical X-ray film (Fuji Film). The silica gel in the radioactive zones was scraped off and counted for radioactivity.

The extracts from the homogenate of treated leaves were concentrated to remove methanol. The resulting aqueous solution was treated as mentioned above for the water washing of the treated leaf.

All of the aqueous solutions remaining after solvent extractions of the treated leaves were combined. Part of the sample (200 ml, 6.58×10^4 dpm) was passed through an Amberlite XAD–4 column (ϕ 3×50 cm), and the column was washed with 800 ml of water. The bound components were eluted with methanol (1 liter) and then with a 0.4% ammonia–acetone mixture (1 : 1, 500 ml). The methanol eluate containing *ca.* 78% of the charged radioactivity was concentrated to dryness under a reduced pressure. Part of the concentrate (9.5 mg, 8820 dpm) was each incubated with cellulase (8 U, ex *Aspergillus niger*, Sigma), pectinase (ex *Aspergillus niger*, Sigma), β–glucosidase (37.6 U, ex Almond, Sigma) and hesperidinase (6 U, *Aspergillus niger*, Sigma) in 15 ml of 0.1 M acetate buffer (pH 4.8) at 38°C for 20 hr. The incubation mixture was extracted twice with 15 ml of dichloromethane at pH 4.8 and then pH 1. The extracts were combined, concentrated and subjected to *tlc* analysis. In this case, the *tlc* plate (Merck 5554®) was developed with *n*–hexane–acetone (7 : 3).

5. Thermal Transformation

ADS and alloxydim placed separately in a capped glass tube were heated at 30, 40 and 50°C in a dark incubator for 20 days. At appropriate intervals, 500 mg of the sample was taken and dissolved in 50 ml of chloroform. The solution (2 μl) was subjected to *tlc* analysis to determine the degradation products. The chloroform solution was extracted twice with 25 ml of 0.5 N sodium hydroxide to remove the intact alloxydim. The chloroform phase was dried over anhydrous sodium sulfate and concentrated. The residue was weighed to know the amount of degradation products (Table 2). The products were confirmed as CM–II and CM–III by *tlc* analysis.

Table 2 Thermal transformation (%) of ADS and alloxydim to the mixture of CM–II and CM–III.

Temperature (°C)	Incubation time (days)			
	ADS	Alloxydim		
	20	7	13	20
30	0	0	0.1	0.1
40	0	0	0.9	1.9
50	0	1.4	3.5	6.2

In another experiment, alloxydim was heated at 120°C for 5 hr or under the same condition as the preparation of CM–II and CM–III to give quantitatively the mixture of CM–II and CM–III at the ratio of 3 to 2.

6. Photochemical Transformation

The methanol solution of ^{14}C–alloxydim (sodium-free ADS) was spotted at the origin

Table 3 Photochemical transformation of ^{14}C–alloxydim on silica gel plate (% of compound detected).

Compounds	Exposure time (min)						Control (Unexposed)
	253.7 nm			365 nm			
	1	5	20	1	5	20	
Alloxydim	94.5	84.0	67.4	95.8	93.7	90.8	98.7
CM–I	1.6	5.9	18.2	0.4	0.6	1.4	0.2
CM–II	0.3	0.7	1.4	0.3	0.4	0.4	0.2
CM–III	0.3	0.8	1.3	0.3	0.5	0.4	0.2
Unknowns	3.3	8.6	11.7	3.2	4.8	7.0	0.7

on *tlc* plate (Merck 5554®). After evaporating the solvent, the plate was placed 8 cm below the UV lamp (100 V, 12 W, Super-Light LS–Bl®, Nikko Sekiei Works) and irradiated with 253.7 or 365 nm light for 1, 5 and 20 min. Then the plate was developed with the solvent system A (Table 1), and the distribution (%) of each compound was calculated by measuring the radioactivity (Table 3).

In another experiment, a solution of alloxydim (1.1 g) dissolved in 200 ml of acetone was irradiated for 5 days at room temperature using a low-pressure mercury ultraviolet lamp in an externally cooled tube. The solvent was evaporated, and the residue was dissolved in chloroform and treated with cold 5% sodium hydroxide solution. The chloroform layer was separated and the solvent was removed *in vacuo* to give CM–I (0.7 g). Unreacted alloxydim (0.2 g) was recovered from the alkaline layer.

7. *Reductive Transformation*

Alloxydim (sodium-free ADS, 3.2 g) in 100 ml of ethanol was hydrogenated over 10% Pd/C (5 g) at ordinary pressure to give CM–I (2.5 g) through the reductive N–O bond dissociation of allyloxyamino moiety.

RESULTS AND DISCUSSION

The behavior of the radioactivity derived from ^{14}C–ADS, when it was applied to the leaf surface of sugar beet plants, is shown in Table 4. Total recovery of the radioactivity gradually decreased with a half life of *ca.* 11 days, and reached *ca.* 31% and 2% of the applied radioactivity after 6 weeks and 6 months, respectively. Most of the recovered radioactivity was retained in the treated leaves during 6 weeks. After this period, the treated leaves gradually died and fell due to natural senescence. The loss of radioactivity from the treated leaves reached 60–70% of the applied one during 28–42 days and may be due to evaporation, because the radioactivity translocated to other part of the plant was less than 6% of the applied one. The calculation of the concentration (ppm) of apparent ADS was based on the radioactivity and the fresh weight of each plant part. Maximum accumulation (4.09 ppm) in the root was observed 4 days after

Table 4 Recovery (%) of radioactivity in sugar beet plant applied with ^{14}C–alloxydim–sodium on the leaves.

Days	Treated leaves					Untreated leaves				Roots			Whole plant	
	W[a]	E[b]	R[c]	W+E+R	C[d]	E	R	E+R	C	E	R	E+R	C	W+E+R	C
0	98.3	0	0	98.3	173	—[e]	—	—	—	—	—	—	—	98.2	173
1	86.4	4.7	0.2	91.3	113	0.3	0	0.3	2.36	0.3	0	0.3	0.53	91.9	49.30
2	73.3	8.0	0.2	81.5	173	0.8	0.1	0.9	9.24	1.0	0	1.0	2.13	83.4	79.56
4	49.8	14.2	0.5	64.5	121	1.5	0	1.5	6.68	1.7	0.1	1.7	4.09	67.7	57.89
7	43.5	14.5	0.9	58.9	87.8	1.3	0.2	1.5	5.92	1.2	0.1	1.3	3.75	61.7	43.14
10	40.7	13.2	0.8	54.7	87.2	1.9	0.2	2.1	9.91	2.4	0.1	2.5	3.48	59.3	37.34
17	23.7	13.9	1.6	39.2	36.0	1.4	0.2	1.6	2.68	2.6	0.2	2.8	1.40	43.6	12.59
21	15.6	16.8	1.7	34.1	22.6	1.1	0.2	1.3	1.41	2.2	0.2	2.4	0.72	37.8	6.42
28	9.2	21.9	1.4	32.5	15.0	1.3	0.4	1.7	1.26	3.6	0.3	3.9	0.34	38.1	2.77
42	5.0	19.0	1.6	25.6	20.8	0.8	0.4	1.2	0.14	3.9	0.5	4.4	0.16	31.2	0.91
120	—	—	—	—	—	—	—	1.1	0.021	—	—	0.9	0.0096	2.0	0.178
180	—	—	—	—	—	—	—	1.0	0.0032	—	—	1.0	0.0020	2.0	0.0025

[a] washing (water+methanol), [b] extract from homogenate, [c] fibrous residue, [d] apparent concentration (ppm) calculated as alloxydim-sodium. [e] These were not counted.

treatment and this concentration diminished to 0.002 ppm after 6 months, whereas the amount in the leaves was 0.0032 ppm.

The radioactivity in the treated leaves was fractionated into the washing, the extract from homogenate and the fibrous residue as shown in Table 5. Until 17 days after the treatment, the amounts of the radioactivity in the washing were larger than those in the extract. As the total radioactivity decreased, the radioactivity in the extract from homogenate increased in comparison with that in the washing, and reached *ca.* 20% of applied radioactivity after 28 and 42 days. This may indicate that the

Table 5 Distribution of radioactivity in sugar beet leaves treated with ^{14}C–alloxydim–sodium (% of radioactivity applied).

Days	Washing				Extract from homogenate			Fibrous residues
	Water washing		MeOH washing	Sub-total	Solvent fraction	Aqueous fraction	Sub-total	
	Solvent fraction	Aqueous fraction						
0	98.2	0.1	0	98.3	—	—	—	—
1	84.9	0.3	1.2	86.4	4.6	0.1	4.7	0.2
2	72.0	0.4	0.9	73.3	7.6	0.4	8.0	0.2
4	45.4	1.8	2.6	49.8	12.0	2.2	14.2	0.5
7	39.9	1.6	2.0	43.5	8.3	6.2	14.5	0.9
10	37.4	1.9	1.4	40.7	9.8	3.4	13.2	0.8
17	18.6	2.6	2.2	23.7	9.6	4.3	13.9	1.6
21	12.2	1.7	1.7	15.6	9.5	7.3	16.8	1.7
28	6.3	1.4	1.5	9.2	11.8	10.1	21.9	1.4
42	2.6	1.6	0.8	5.0	8.8	10.2	19.0	1.6

Table 6 Balance of ^{14}C–compounds in solvent extracts of leaves applied with ^{14}C–alloxydim–sodium (% of radioactivity applied).

	Days									
	0	1	2	4	7	10	17	21	28	42
In washing										
Alloxydim	88.6	74.0	56.4	26.1	17.7	4.8	0.1	0	0	0
CM–I	0.6	3.6	6.0	9.1	9.3	17.3	10.6	7.6	3.4	1.1
CM–II	0.6	0.7	1.2	1.3	2.3	1.2	0.2	0.1	0	0
CM–III	1.3	0.7	0.9	1.1	1.8	1.3	0.7	0.1	0	0
Others	7.1	7.1	8.4	10.4	10.8	14.2	9.5	6.0	4.6	2.3
Total	98.2	86.1	72.9	48.0	41.9	38.8	21.1	13.9	7.8	3.4
In extract from homogenate										
Alloxydim	0	3.3	4.3	4.6	2.1	0.8	0.3	0.1	0.4	0.3
CM–I	0	0.3	0.6	1.8	0.7	1.7	1.1	1.5	1.0	0.9
CM–II	0	0.2	0.2	1.0	0.6	1.4	0.9	0.4	0.3	0.3
CM–III	0	0.2	0.6	1.0	0.6	1.2	0.8	0.6	0.3	0.3
Others	0	0.6	1.9	3.6	4.3	5.7	6.5	6.9	9.8	7.0
Total	0	4.6	7.6	12.0	8.3	9.8	9.6	9.5	11.8	8.8
Sum of washing and extract from homogenate										
Alloxydim	88.6	77.3	60.7	30.7	19.8	5.6	0.4	0.1	0.4	0.3
CM–I	0.6	3.9	6.6	10.9	10.0	18.0	11.7	9.1	4.4	2.0
CM–II	0.6	0.9	1.4	2.3	2.9	2.6	1.1	0.5	0.3	0.3
CM–III	1.3	0.9	1.5	2.1	2.4	2.5	1.5	0.8	0.3	0.3
Others	7.1	7.7	10.3	14.0	15.1	19.9	16.0	12.9	14.2	9.3
Total	98.2	90.7	80.5	60.0	50.2	48.6	30.7	23.4	19.6	12.2

radioactivity remaining on the leaf (*i.e.* washing) dissipated easily and that once permeated into the leaf (*i.e.* extracts and fibrous residue) persisted till the treated leaf fell. In the washing, most of the radioactivity was recovered with water and extractable with organic solvent. The unextractable radioactivity in the fibrous residue was less than 2% of the applied radioactivity during all sampling times.

Concerning the metabolites in the plants, organosoluble ^{14}C–compounds were analyzed by means of *tlc* cochromatography. The balance of ^{14}C–compounds in the treated leaves is listed in Table 6. Within a week, the main ^{14}C–compound was ADS or alloxydim in the washings and in the extracts from homogenate. After 10 days, it was replaced by CM–I, which persisted as the main metabolite until 42 days. The amount of CM–I for applied ADS reached 18.0% at 10 days and decreased to 2.0% at 42 days. Other identified metabolites were CM–II and CM–III with 2.6% and 2.5% of applied radioactivity at 10 days and 0.3% and 0.3% at 42 days, respectively. Other compounds such as CM–IV, V, VI and VII, VIII were not detected in this experiment. A few unknowns including the substances remaining on the origin of *tlc* were found, but in small amounts. They also decreased with time.

^{14}C–Components in the water-soluble fractions remaining after the solvent extractions increased with time and reached 11.8% of the applied radioactivity at 42 days. The purification of ^{14}C–components was tried by an Amberlite XAD–4 column. The methanol fraction of the XAD–4 purification accounted for 77% of the radioactivity placed on the column. The water and ammonia-methanol fractions contained 20 and 3% of the charged radioactivity, respectively. Direct *tlc* analysis of the methanol eluate gave poor separation of ^{14}C–spots. The ^{14}C–components in the eluate were tested for hydrolysis with several enzymes as used in published studies on plant metabolism.[5–7] In the case of β–glucosidase and hesperidinase, the extraction rate with ethyl acetate was nearly the same (about 10%) with or without enzyme treatment. As compared with above results, cellulase and pectinase gave a better rate of the solvent extraction (about 40%). The autoradiogram of the developed *tlc* plate showed that about 60% of the radioactivity in the extract corresponded to CM–I and other ^{14}C–spots were mainly found at the *tlc* origin. Therefore it can be concluded that CM–I is one of the main aglycones present in the water soluble fraction. Still and Mansager[5] said in their report on alfalfa metabolism of propham that, amongst enzymes tested for hydrolysis of polar metabolites, cellulase was shown to yield the highest conversion to the aglycone, which was proved as 4–hydroxypropham. Zurqiyah *et al.*[6] used an enzyme mixture including cellulase, β–glucosidase and hesperidinase to get the highest cleavage of conjugates derived from propham. Thus cellulase seems to be an important enzyme to hydrolyse conjugates obtained from plant. But the action of this enzyme is nonspecific, and therefore, the enzyme provides little information on the nature of the conjugates.

The radioactivity in other parts of the plant except the treated leaves was so little (Table 4) that this was not analyzed for metabolites.

According to the above tracer experiment and *tlc* cochromatography, ADS decreased with time to give CM–I, CM–II and CM–III in the leaves. Concerning the mechanism of transformation of ADS, a reductive dissociation may be involved to give CM–I and a Beckmann rearrangement reaction coinciding with an intramolecular cyclization may be required to give CM–II and CM–III. ADS is a sodium salt of an acid, alloxydim, having pK_a' of 3.7, which is present as the monoanion and/or the acid in aqueous solution, and the possibility of many tautomeric forms should be considered to understand the transformations. CM–I was produced by catalytic reduction as described in the METHOD and seems to occur by photoreduction on plant or silica gel plate (Table 3). The mechanism of the formarion of CM–II and CM-III can be explained as follows. In certain tautomeric isomers, the cyclohexane ring of alloxydim is coplaner with the six membered ring which is formed by hydrogen bonding and the allyloxy group should be in the anti position against the cyclohexane group on the C=N bond.[8] Therefore, the Beckmann rearrangement reaction occurs, which coincides with the intramolecular cyclization to form the oxazoles as the reactions of the quinolone

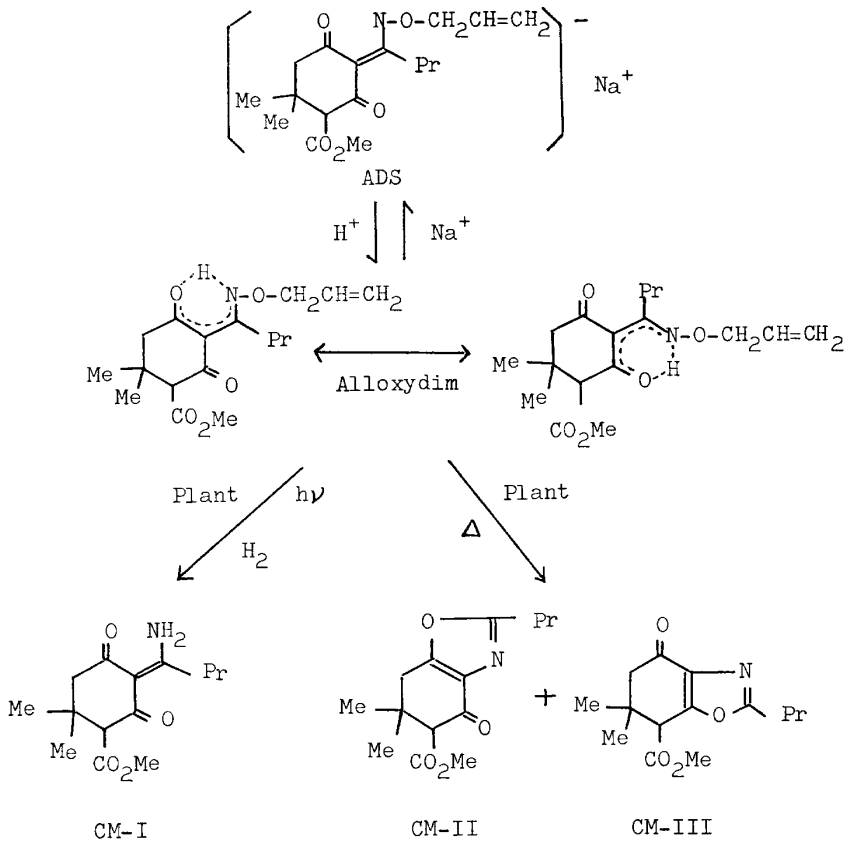

Fig. 1 The schematic pathway for the transformation of ADS in plant and under other conditions.

oxime[8] and a pyridone or dehydroacetic acid oxime.[9] This type of reaction occurs under various thermal conditions, even at lower temperature (Table 2).

The schematic pathway for the transformation of ADS in plant and under other conditions is summarized in Fig. 1. The participation of any plant enzyme in these reactions was not clear in this experiment and should be studied by further investigations.

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REFERENCES

1) M. Sawaki, I. Iwataki, Y. Hirono & H. Ishikawa: U.S. Pat. No. 3950420, 1976; 4011256, 1977
2) Y. Hirono, H. Ishikawa, T. Naohara & H. Hosaka: Abstract of the 1st Annual Meeting of Pesticide Science Society of Japan, Tokyo, March, No. 240, 1976
3) I. Iwataki & Y. Hirono: Abstract of the Symposium IIb in the 4th International Congress of Pesticide Chemistry (IUPAC), Zürich, Switzerland, July, P. II–12, 1978; "Advances in Pesticide Science," Pergamon Press, p. 235, 1979
4) Y. Hashimoto, K. Ishihara & Y. Soeda: Abstract of the 3rd Annual Meeting of Pesticide Science Society of Japan, Tokyo, March, No. 118, 1978
5) G. G. Still & E. R. Mansager: *Pestic. Biochem. Physiol.* **5**, 515 (1975)
6) A. A. Zurqiyah, L. S. Jordan & V. A. Jolliffe: *Pestic. Biochem. Physiol.* **6**, 35 (1976)
7) T. R. Roberts: *Pestic. Biochem. Physiol.* **7**, 378 (1977)
8) B. Staskum: *J. Org. Chem.* **31**, 2674 (1966)
9) T. Kato, J. Kawamata & T. Shibata: *J. Pharm. Soc. Japan* **88**, 106 (1968)

要　　約

除草剤アロキシジンのサトウダイコンにおける運命

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新除草剤アロキシジン (alloxydim-sodium, ADS) の植物における代謝分解を，その化学的変換とともに研究した．^{14}C 標識 ADS をサトウダイコンの葉に処理し，野外条件下におくと初期の半減期 11 日で放射能が揮散した．処理葉以外への浸透，移行は少なく，6 カ月後の根における ADS 換算濃度は，0.002 ppm であった．処理葉における代謝分解物はおもにアミノ体 (CM-I) で，その他は少量のオキサゾール体(CM-II および CM-III) であった．10 日後における代謝分解物の生成量は，処理量に対しそれぞれ CM-I 18.0%，CM-II 2.6%，CM-III 2.5% であった．これらの化合物が生成する要因を検討したところ，CM-I は光により還元的分解で，またオキサゾール体は熱によりベックマン転移して生成するものと考えられた．