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The Products of Metabolism of [^{14}C]Triadimefon in the Grain and in the Straw of Ripe Barley

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Little was known about the metabolism of the fungicide triadimefon in plants (Fig. 1); the metabolite triadimenol had been identified (CLARK et al. 1978, FISHER et al. 1979, GASZTONYI & JOSEPOVITS 1979, VON SPECHT 1977). More recently we studied the metabolism of the ^{14}C labelled triadimefon in barley shoots, and detected triadimefon, and the free and conjugated triadimenol and 4-chlorophenol (ROUCHAUD et al. 1981). In the present work, we studied the metabolism products of triadimefon in the grains and the straw of ripe barley which had been treated with [^{14}C] triadimefon.

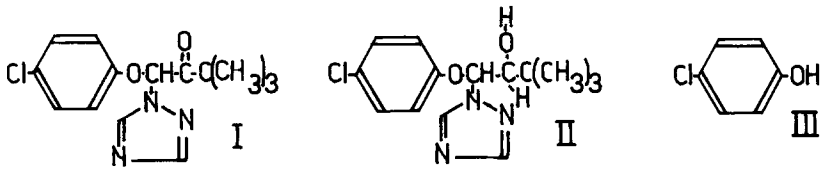

Fig. 1. Triadimefon (I), triadimenol (II), and 4-chlorophenol (III).

MATERIALS AND METHODS

Chemicals, and physical and chemical methods of analysis. [^{14}C]Triadimefon was uniformly labelled in the phenyl ring (0.88 mCi/mmol) (ROUCHAUD & MEYER 1981). Bayleton 25WP (wettable powder containing 25% by weight of triadimefon) was obtained from Bayer, Belgium. Thin-layer and gas-liquid chromatographies, radioactivity measurements, mass spectrometry, and acetylation of the phenol residues were made as described (ROUCHAUD et al. 1981.)

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Plant culture and treatment. Barley plants (cv. Hebe) were sowed (30 pots, 33 plants/pot; 30/10/80) in plastic pots (25 cm diameter), containing a mixture of Rhine sand and expanded perlite (1/1, v/v). The soil was drenched with the nutritive Shive and Robbin's solution I (JOHNSON et al. 1959), iron (III) sodium ethylenediaminetetraacetate (0.2 g/litre) being used in the place of iron (III) sulphate. The plants were grown in a greenhouse (day: 16 h, 10 000 lux, 15°C before the first treatment, after the first treatment the temperature was progressively risen so that 2 months after the first treatment it was 20°C; night, 15°C). Spraying with water of the aerial part was avoided. At growth stage 6 (stem extension, first node of stem visible at base of shoot; 24/12/80), the plants were treated by spraying an emulsion of a mixture of 83 mg of [^{14}C]triadimefon (0.25 mCi) and 111 mg of Bayleton 25WP in 1.7 litre of water containing, relatively to the water, 0.1% by weight of Tween^{R} 80 (polyoxyethylensorbitanmonooleat, Merck). This corresponded to a rate of 500 g of triadimefon ha^{-1} which was four times higher than the recommended one. Spraying was made with a small hand-apparatus, and at a short distance from the plants so that the losses of pesticide solution were minimized. At growth stage 10, i.e. 66 days posttreatment, half of the shoots were harvested, and their analysis has been described (ROUCHAUD et al. 1981).

At growth stage 10.1 (heading, the awns just showing; 10/3/81, i.e. 76 days after the first treatment) the remaining plants (15 pots) were treated a second time at a dose equal to that of the first treatment, thus spraying an emulsion of a mixture of 41.5 mg of [^{14}C]triadimefon (0.125 mCi) and of 55.5 mg of Bayleton 25WP. At growth stage 11.4 (ripe for cutting, straw dead; 42 and 118 days after respectively the second and the first treatment), the plants were harvested, and the grains and the straw were analysed separately.

Extraction of the grains and of the straw. The concentration of the radioactivity in the grains was 0.99.10^{4} disintegrations min^{-1}g^{-1} fresh weight. The grains were washed rapidly and successively with water and methanol; these washings were discarded as they did not contain a significant amount of radioactivity. After air-drying the grains were milled, the flour (100 g) was macerated (2 h, 20°C) in water (100 ml), the mixture was homogenized with acetone (400 ml) in a Sorvall omnimixer (20°C, 5 min, 8000 rev min^{-1}), centrifugation (10 min, 5000 rev min^{-1}) gave a solid residue which was extracted two times more with a mixture of acetone+water, 3.3+1 (2 x 400 ml) (Fig. 2). The whole procedure was repeated with 100 g of fresh flour, the extracts and insoluble residues were gathered with the ones of the preceding extraction, giving respectively the Extract 1 and Solids 1 from the extraction of 200 g of grains which were analysed further.

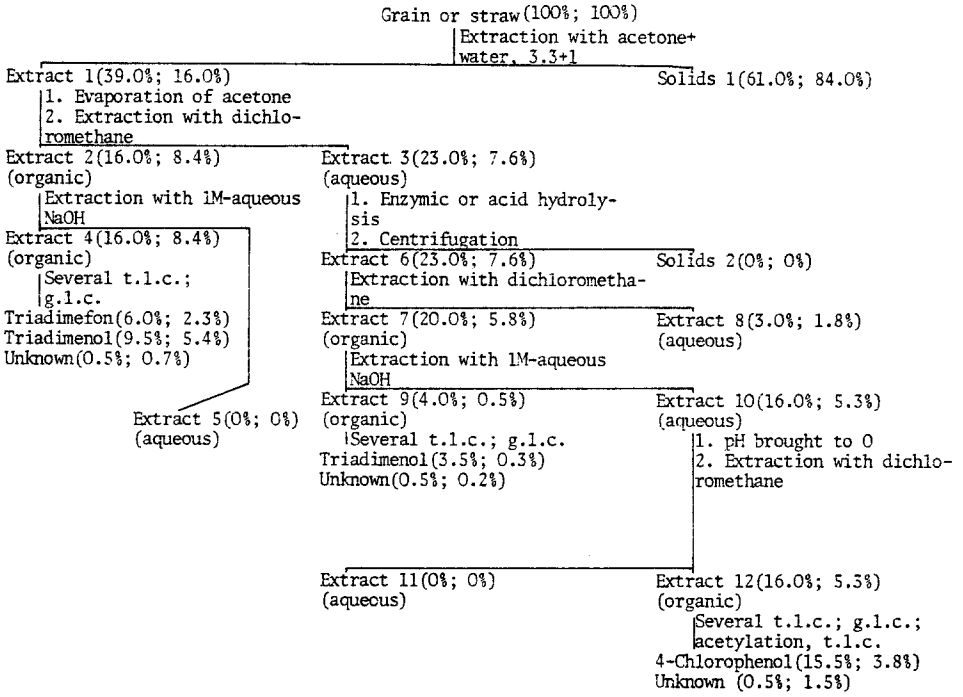

Fig. 2. Common fractionation scheme for the grains and the straw of ripe barley. The respective percentages give the distribution of ^{14}C among the extracts and solids; the percentages for the fractionation of the grains are relative to the total ^{14}C incorporated into the grains; the percentages for the fractionation of the straw are relative to the total ^{14}C incorporated into the straw.

The concentration of the radioactivity in the straw was $14.9.10^4$ disintegrations min^{-1} g^{-1} fresh weight. The straw was washed rapidly and successively with water and methanol; these washings were discarded as they did non contain a significant amount of radioactivity. After air-drying the straw was milled, the powder (100 g) was macerated and extracted in the same way as the flour from grain, giving the final Extract 1 and Solids 1 from the extraction of 100 g of straw, which were analysed further.

Acetone was evaporated under vacuum at 40°C from Extract 1, the aqueous solution was extracted three times with dichloromethane (3 x 700 ml and 3 x 400 ml respectively for the extracts from the grains and from the straw), the organic and aqueous layers were separated, each was concentrated under vacuum (40°C, rotavapor) to 100 ml, giving the dichloromethane Extract 2 and the aqueous Extract 3.

Extract 2 was extracted with aqueous 1M-sodium hydroxi-

de, giving the dichloromethane Extract 4 and the aqueous Extract 5.

Extract 3 was submitted to an enzymic or an acid deconjugation hydrolysis.

For the enzymic hydrolysis, an aliquot of Extract 3 was taken up in 0.1M-sodium acetate buffer (pH 5.0, 20 ml). β-Glucosidase (almonds; BDH Chemicals Ltd; 0.2 g) and a drop of toluene were added, and the mixture was incubated at 35°C for 24 h with magnetic stirring. The precipitate (Solids 2) was separated by centrifugation; the supernatant liquid (Extract 6) was extracted three times with dichloromethane (3 x 100 ml), and the organic layer (Extract 7) and aqueous layer (Extract 8) were separated.

For the acid hydrolysis, most of Extract 3 was dissolved in aqueous 2M-hydrochloric acid (50 ml), heated at 80°C for 3 h with stirring, and the hydrolysate was treated in the same way as the one from the enzymic hydrolysis.

Extract 7 was extracted with aqueous 1M-sodium hydroxide, giving the dichloromethane Extract 9 and the aqueous Extract 10 solutions. The pH of Extract 10 was brought to 0 by means of concentrated hydrochloric acid, the solution was extracted with dichloromethane, giving the aqueous Extract 11 and the dichloromethane Extract 12 solutions.

RESULTS

When triadimefon, triadimenol, and 4-chlorophenol were separately added to the grains or the straw from control plants (which had not been treated with triadimefon), so that their concentrations were 0.1 and 0.5 mg kg^{-1} fresh weight respectively in the grains and in the straw, triadimefon and triadimenol were recovered in Extract 4 with the respective recoveries of 75±5 and 73±6% (two assays for each compound, as for each of the further mentioned recovery experiments) in the grains; 82±5 and 81±6% in the straw. In Extract 5 from the grains and from the straw, respectively 72±6 and 81±7% of the 4-chlorophenol were recovered.

When triadimefon, triadimenol, and 4-chlorophenol were separately added to Extract 3 from untreated plants, at concentrations corresponding to 0.1 and 0.5 mg kg^{-1} fresh weight respectively in the grains and in the straw, triadimefon and triadimenol were found with the respective recoveries of 76±8 and 74±9% in the Extract 9 from the grains; 82±5 and 88±7% in the Extract 9 from the straw. 73±7 And 78±7% of the 4-chlorophenol were recovered in Extract 12 respectively obtained from the grains and the straw. The enzymic and acid hydrolyses gave similar recoveries, and also

similar results for the analysis of the treated plants. The recovery experiments showed that none of these compounds was decomposed into an other one during the analytical procedure, and none of them was found into an extract other than the indicated one.

Five completely separated extractions and analyses were made of the plants from five different pots; the results are the mean values and Tables 1 and 2 give the standard deviations.

Analysis of Extract 4. Extract 4 was t.l.c. with ammonia +ethanol, 1+1, the main radioactive band (R_F=0.92--1.0) was extracted, the extract was t.l.c. with chloroform, giving mainly the broad radioactive Band 1 (R_F=0.28-0.36) and the radioactive Band 2 (R_F=0.50). Band 1 was extracted, t.l.c. with ethyl acetate; both the radioactive bands (R_F=0.76 and 0.86) were extracted and t.l.c. with methanol; the radioactive bands (R_F=0.68 and 0.80) of triadimenol were extracted and t.l.c. with the solvent system benzene+dioxane+acetic acid, 90+25+4; the radioactive bands of triadimenol (R_F=0.55 and 0.68) were extracted and analysed.

Band 2 was extracted, t.l.c. with ethyl acetate, the radioactive band (R_F=0.85) corresponding to triadimefon was extracted and t.l.c. with toluene; the radioactive band (R_F= 0.28) of triadimefon was extracted and t.l.c. with the solvent system benzene+dioxane+acetic acid, 90+25+4; the radioactive band (R_F=0.78) of triadimefon was extracted and analysed.

The g.l.c. of the respective purified extracts from the radioactive Bands 1 and 2 showed that they contained triadimenol (a mixture of both diastereoisomers) and triadimefon; m.s. also confirmed the presence of these compounds in the extracts from the straw. Measurements of the radioactivity and of the g.l.c. peaks indicated the percentages of radioactivity corresponding to these compounds and their concentrations in the straw and in the grains (Fig. 2, Tables 1 and 2).

Analysis of Extract 9. Extract 9 was purified by successive t.l.c., extracting after each t.l.c. the radioactive bands corresponding to both diastereoisomers of triadimenol and again t.l.c. this extract; this was made in the same way as with Extract 4 and its corresponding radioactive Band 1. The g.l.c. of the purified extract showed that it contained the mixture of both diastereoisomers of triadimenol. Measurements of the radioactivity and of the g.l.c. peaks indicated the percentages of radioactivity corresponding to triadimenol and its concentration in the straw and in the grains (Fig. 2, Tables 1 and 2). In the plant, the residue of triadimenol was conjugated to plant constituents.

TABLE 1

Distribution of the radioactivity respectively incorporated into the grains and into the straw, among triadimefon and its metabolites. For the grains, the percentages of radioactivity are relative to the total ^{14}C incorporated into the grains; for the straw, the percentages of radioactivity are relative to the total ^{14}C incorporated into the straw. Data are the means ± s.d. for five determinations.

Compound	Acetone+water (3.3+1) soluble		Acetone+water (3.3+1) insoluble	Total
	Free compounds	Conjugated compounds		
	1. In the grains			
Triadimefon	6.0±0.5			6.0
Triadimenol	9.5±1.0	3.5±0.6		13.0
4-Chlorophenol	0.0±0.0	15.5±1.2		15.5
Unknown	0.5±0.2	4.0±0.5	61.0±5.1	65.5
Total	16.0	23.0	61.0	100
	2. In the straw			
Triadimefon	2.3±0.3			2.3
Triadimenol	5.4±0.6	0.3±0.1		5.7
4-Chlorophenol	0.0±0.0	3.8±0.8		3.8
Unknown	0.7±0.2	3.5±0.4	84.0±7.8	88.2
Total	8.4	7.6	84.0	100

TABLE 2

Concentration of triadimefon and of its metabolites in the grain and in the straw of ripe barley (mg kg^{-1} fresh weight; data are the means ± s.d. for five determinations).

	Free compounds	Conjugated compounds	Total
	1. In the grains		
Triadimefon	0.12±0.01	0.00±0.00	0.12
Triadimenol	0.19±0.02	0.07±0.01	0.26
4-Chlorophenol	0.00±0.00	0.13±0.01	0.13
	2. In the straw		
Triadimefon	0.70±0.09	0.00±0.00	0.70
Triadimenol	1.61±0.18	0.10±0.03	1.71
4-Chlorophenol	0.00±0.00	0.49±0.10	0.49

Analysis of Extract 12. Extract 12 was purified by successive t.l.c. in the same way as Extract 9; at each t.l.c. the main radioactive band corresponded to 4-chlorophenol. The purified extract was analysed by g.l.c. before and after acetylation; the extract from the straw was analysed by m.s. Each of these methods indicated that Extract 12 contained 4-chlorophenol which, in the plant, was conjugated to plant constituents. Measurements of the radioactivity and of the g.l.c. peaks indicated the percentages of radioactivity corresponding to 4-chlorophenol and its concentration in the straw and in the grains (Fig. 2, Tables 1 and 2). In the plant, this residue of 4-chlorophenol was conjugated to plant constituents.

DISCUSSION

The results are relative to the analysis of the grains and of the straw of ripe barley harvested 42 days after the second treatment with [^{14}C]triadimefon made at the growth stage at which the awns were just showing, and 118 days after the first treatment made when the first node of the stem was visible at base of shoot; at each treatment, the dose was four times higher than the recommended one. 14% Of the sum of the applied radioactivity was found incorporated into the aerial part of the plants (grains plus straw). 4 And 96% of the total radioactivity, incorporated into the aerial part of the harvested plants, were incorporated respectively into the grains and into the straw. The concentrations of [^{14}C]triadimefon equivalents were 2.0 and 30.0 mg kg^{-1} fresh weight respectively in the grains and in the straw.

In the grains, 61% of the incorporated radioactivity was insoluble in acetone+water, 3.3+1 (Tables 1 and 2). 16% Of the radioactivity of the grains was made up of soluble compounds which were free in the grains, mainly triadimefon and triadimenol at the respective concentrations of 0.12 and 0.19 mg kg^{-1} fresh weight in the grains. 23% Of the radioactivity of the grains was soluble and made up of compounds which, in the grains, were conjugated to plant constituents; these conjugated compounds were mainly triadimenol and 4-chlorophenol at the concentrations of 0.07 and 0.13 mg kg^{-1} fresh weight in the grains.

In the straw, 84% of the incorporated radioactivity was insoluble in acetone+water (Tables 1 and 2). 8.4% Of the radioactivity of the straw was made up of free soluble compounds, mainly triadimefon and triadimenol at the concentrations of 0.70 and 1.61 mg kg^{-1} fresh weight in the straw. 7.6% Of the radioactivity in the straw was made up of soluble compounds conjugated to plant constituents, mainly 4-chlorophenol at the concentration of 0.49 mg kg^{-1} fresh weight in the straw.

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