Metabolism of the Aryloxyphenoxypropanoate Herbicide, CGA 184927, in Wheat, Barley and Maize: Differential Effects of the Safener, CGA 185072*

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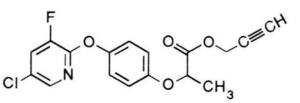
Aryloxyphenoxypropanoate Herbicide, Safener, Herbicide Metabolism, Monocotyledonous Crops, Glycosyl Conjugation

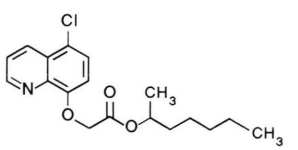
The influence of the safener, CGA 185072 (5-chloro-8-quinolinoxy-acetic acid-1-methyl-hexyl-ester), on the metabolism of the aryloxyphenoxypropanoate herbicide, CGA 184927 (2-propynyl-*R*-2-[4-(5-chloro-3-fluoro-2-pyridinyloxy)-phenoxy]-propionate), was studied in excised leaves of wheat, barley, and maize. In wheat and barley, CGA 184927 readily underwent ester hydrolysis followed by hydroxylation at the pyridinyl moiety as well as ether cleavage between the pyridinyl and the phenyl ring. Ether cleavage constituted the minor pathway in both species. All metabolites were subject to glycosyl conjugation. Tetcyclacis strongly inhibited pyridinyl-ring hydroxylation in wheat. Metabolism by hydroxylation and ether cleavage was more rapid in wheat than in barley, and was found to be accelerated in the presence of the safener CGA 185072 in both wheat and, to a lesser degree, in barley. Moreover, the safener increased the capacity for O-glycoside formation in wheat as suggested from studies using the ^{14}C-labelled pyridinyl-ring hydroxylated metabolite as a precursor. In maize, which is highly susceptible to CGA 184927, rapid ester hydrolysis of CGA 184927 and partial conversion of the corresponding carboxylic acid to glycosyl ester conjugate(s) occurred. However, no further transformation of the herbicide was found in maize, both in the absence or presence of CGA 185072. It is concluded that the ability of CGA 185072 to protect wheat from injury by the herbicide, CGA 184927, and to confer partial protection to barley, is related to the ability of the safener to stimulate herbicide metabolism in these crop species.

Introduction

The herbicide and safener combination, CGA 184927 + CGA 185072 (for structures see Fig. 1), has recently been introduced for post-emergent control of annual grasses in small grain cereals including wheat, rye and triticale [1, 2]. CGA 184927 (2-propynyl-*R*-2-[4-(5-chloro-3-fluoro-2-pyridinyloxy)-phenoxy]-propionate) is an aryloxyphenoxypropanoate-type herbicide that is not tolerated in cereal crops when used alone. The safener CGA 185072 (5-chloro-8-quinolinoxy-acetic acid-1-methyl-hexyl-ester), applied as a tank mixture with



CGA 184927



CGA 185072

Fig. 1. Chemical structures of CGA 184927 (herbicide) and CGA 185072 (safener).

CGA 184927, selectively protects wheat, rye and triticale from herbicidal injury. The safener does not affect the control of grass weeds such as *Alopecurus myosuroides, Avena* spp., *Lolium* spp., *Phalaris* spp., *Poa trivialis* and *Setaria* spp. [1]. Barley exhibits a higher susceptibility to CGA 184927 as compared to wheat, and CGA 185072 does not confer complete protection to barley although some safening effects are obtained [2]. Maize, on the other hand, is highly susceptible to CGA

Abbreviations: BAS 145138, 1-dichloroacetyl-hexahydro-3,3-8 a-trimethyl-pyrrolo-[1,2-a]-pyrimidin-6-(2 H)-one; chlorimuron ethyl, 2-[3-(4-chloro-6-methoxypyrimidin-2-yl)-ureidosulfonyl]-benzoic acid ethylester; diclofop, 2-[4-(2,4-dichlorophenoxy)-phenoxy]-propionate; tetcyclacis, 5-(4-chlorophenyl)-3,4,5,9,10-pentaaza-tetracyclo-[5,4,1,$0^{2,6}$,$0^{8,11}$]dodeca-3,9-diene.

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184927 and no protective action of the safener has been observed [3].

In recent years, evidence has been provided that safener-induced enhancement of herbicide metabolism in protected plants is involved as a major mechanism of action of the currently developed safeners [4]. The purpose of the present study was to compare the initial metabolism of the herbicide, CGA 184927, in excised leaves of wheat, barley and maize and to investigate the effects of the safener, CGA 185072, on herbicide metabolism in these monocotyledonous crop species.

Materials and Methods

[Phenyl-U-^{14}C]CGA 184927 (sp. act. 633 MBq/mmol, radiochemical purity 99%), nonlabelled CGA 184927, CGA 185072, and reference standards mentioned in the text were synthesized in the laboratories of Ciba-Geigy Ltd., Basle, Switzerland. Cellulase from *Aspergillus niger* was obtained from Sigma (St. Louis, Mo.).

Spring wheat (*Triticum aestivum* L. *cv.* Besso), spring barley (*Hordeum vulgare* L. *cv.* Cornel), and maize (*Zea mays* L. *cv.* Blizzard) were grown in vermiculite and supplied with water (maize) or with Hewitt nutrient solution containing a 2-fold concentration of KNO_3. Plants were grown under a light intensity of 1600 lux with a 16 h photoperiod, at 24 °C, 70% relative humidity for 8 days (wheat and barley) or at 27 °C, 80% relative humidity for 7 days (maize). For the metabolism studies, the second leaves of plants were excised under water and placed with the cut ends into an aqueous solution containing 10% (v/v) methanol, 30 μM [^{14}C]CGA 184927 and, were indicated, 10 μM CGA 185072. After allowing a 2 h uptake period, the leaves were transferred to quarter-strength Hewitt solution and returned to the growth chamber for 6 h. Total [^{14}C]uptake was not altered by CGA 185072. The leaves were then frozen on dry ice, pulverized, and extracted three times with a 10:1 (v/w) ratio of 80% acetonitrile using an Ultra-Turrax homogenizer. [^{14}C]Recovery from the residual treating solutions and leaf extracts was in excess of 96%. The extracts were evaporated *in vacuo*, redissolved in 80% methanol, and subjected to thin-layer chromatography (TLC) before and after digestion with cellulase (EC 3.2.1.4) according to [5]. Acid hydrolysis of conjugates was performed in 6 M HCl at 50 °C for 14 h. Following enzymatic or acid hydrolysis, the samples were passed over BondElut C-18 cartridges (Analytichem International, Harbor City, Cal.) and bound radioactivity eluted with 80% methanol ([^{14}C]recovery from this step ≥97%). Experiments were repeated at least three times and representative data are presented.

Radiolabelled metabolites were initially separated by two-dimensional TLC on silica gel 60 F_{254} plates (Merck) first in toluene:ethyl acetate:formic acid (83:14:3, v/v) followed by $CHCl_3$:methanol:acetone:formic acid:H_2O (55:20:18:2:1, v/v), and thereafter routinely by one-dimensional TLC in the former solvent system. [^{14}C]Products on two-dimensional plates were detected with a Raytest radiochromatogram camera, and nonlabelled reference compounds were visualized under UV light (254 nm). Radioactive zones on one-dimensional plates were localized and quantified with a Berthold LB 284 linear analyzer. HPLC was performed on a 5 μm Nucleosil C-18 column (4.6 × 250 mm) which was connected to a UV-detector (240 nm) and a Berthold LB 507 A radiochromatography monitor. The column was eluted at 1 ml/min with a linear H_2O/acetonitrile (both containing 0.1% TFA) gradient from 10% to 80% over 20 min, followed by isocratic elution at 80% for 10 min. Metabolites were characterized by co-chromatography on two-dimensional TLC and HPLC with the authentic reference standards **1** and **3** (for structures see Fig. 2). The structure of metabolite **2** from wheat cells has previously been established by mass spectrometry (MS) and ^{1}H NMR (J. Stingelin, unpubl. results). The corresponding metabolites from excised wheat and barley leaves were chromatographically indistinguishable from metabolite **2.** The metabolite from excised wheat leaves was purified by TLC and HPLC, methylated with CH_2N_2, and analyzed by MS using the previously described instrumentation [6]. The electron impact mass spectrum was closely analogous to that of authentic methylated **2.**

Results and Discussion

Greenhouse studies have demonstrated that the herbicide, CGA 184927, is most active when ap-

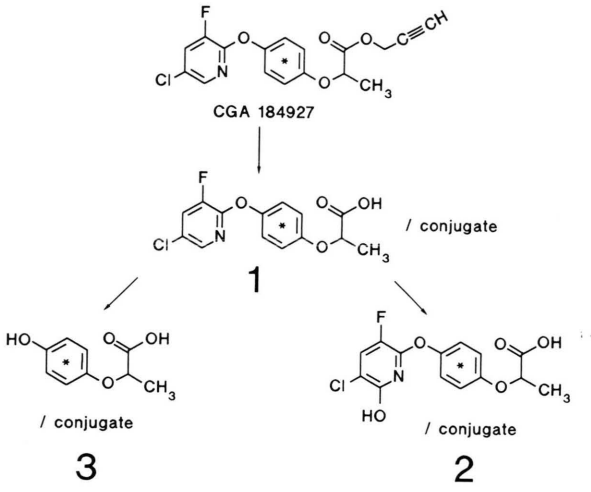

Fig. 2. Proposed metabolic pathway of [phenyl-U-^{14}C]-CGA 184927 in excised leaves of wheat and barley. The ^{14}C-labelled ring is denoted by an asterisk. The position of hydroxylation of **2** in barley has not been determined.

plied to the plant foliage [1]. The safener, CGA 185072, must also be applied to the leaves to provide full protection to cereal crops. In the present report, therefore, we used excised leaves of wheat, barley, and maize to study safener effects on herbicide metabolism. Leaves were pulse-treated for 2 h with [^{14}C]CGA 184927, with or without CGA 185072 added to the treatment solution, and then allowed to metabolize the herbicide for 6 h prior to the analysis of metabolite patterns. In wheat, ester hydrolysis of CGA 184927 to the carboxylic acid (**1,** for structures see Fig. 2) was apparently rapid, and no CGA 184927 parent ester was detectable within the leaf tissue immediately after pulse-application (data not shown). The free acid forms are generally considered to constitute the herbicidal active species of aryloxyphenoxypropanoic acid esters [7]. The herbicide acid (**1**) was extensively metabolized in wheat to both the pyridinyl-ring hydroxylated derivative (**2**) and the ether cleavage product (**3,** Fig. 3A). Metabolites **2** and **3** were present mainly in the form of glycosyl conjugates, whereas the herbicidal acid (**1**) was only partly conjugated, presumably as sugar ester(s). 10 to 15% of the total radioactivity extracted from wheat leaves remained in an unidentified polar fraction. In the presence of the safener, CGA 185072, herbicide metabolism was found to be

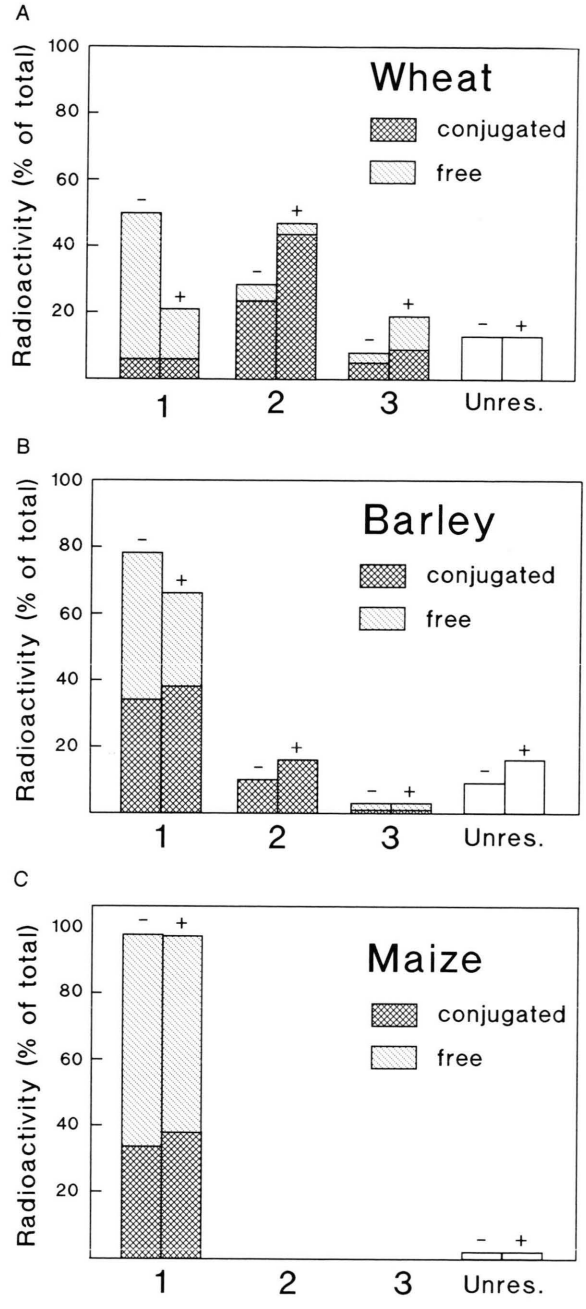

Fig. 3. Effect of the safener, CGA 185072, on the metabolism of CGA 184927 in wheat (A), barley (B), and maize (C). Excised leaves were fed [phenyl-U-^{14}C]CGA 184927 for 2 h in the absence (−) or presence (+) of CGA 185072 and thereafter allowed to metabolize [^{14}C]CGA 184927 for 6 h. Total radioactivity extracted from the leaves was set 100%. See Fig. 2 for key to metabolites. Unres. = polar metabolites not resolved on TLC.

enhanced. Both, the pyridinyl-ring hydroxylated metabolite (**2**) as well as the ether cleavage product (**3**) had increased. In barley, the pattern of metabolites formed from CGA 184927 was found to be qualitatively similar to that from wheat (Fig. 3 B). CGA 184927 was rapidly hydrolyzed to **1,** which formed a polar acid-labile conjugate(s), presumably a glycosyl ester(s). This conjugate(s) was not further characterized in this study. The rate of pyridinyl-ring hydroxylation to **2** and ether cleavage to **3,** however, was found to be considerably slower in barley as compared to wheat. The safener, CGA 185072, only slightly stimulated the hydroxylation of **1** to **2** in barley. In maize, rapid ester hydrolysis of CGA 184927 to **1** and partial conversion to a glycosyl ester conjugate(s) occurred (Fig. 3C). However, in the short-term metabolism studies described here, no further transformation of the herbicide was found in excised maize leaves, both in the absence and in the presence of the safener, CGA 185072.

Recently, arylhydroxylation of the related herbicide, diclofop, has been shown to be catalyzed in wheat by a cytochrome P450-dependent monooxygenase [8, 9]. This monooxygenase reaction was inhibited by the cytochrome P450 inhibitor, tetcyclacis. In excised wheat leaves, pyridinyl-ring hydroxylation of **1** to **2** was found to be strongly inhibited in the presence of tetcyclacis (Fig. 4). This result provides indirect evidence for the involvement of a cytochrome P450 monooxygenase in the hydroxylation of CGA 184927 in wheat. It furthermore suggests that the safener, CGA 185072, exerts its effect on a cytochrome P450 enzyme, as has recently been demonstrated for various safeners in wheat and maize [6, 10–12].

In addition, the safener CGA 185072 was found to accelerate the conjugation of metabolite **2** (Table I). For this experiment, [^{14}C]labelled **2** was isolated from wheat leaves and its identity verified by MS of the methylated derivative. [^{14}C]Labelled **2** was then fed to excised wheat leaves and the extent of conjugation in the absence and in the presence of the safener was determined by HPLC. Two different conjugates of yet unknown structure, conjugate **I** and **II,** were readily formed. Based on the high susceptibility to β-glucosidase (EC 3.2.1.21), conjugate **II** is assumed to be an O-glycoside of **2.** The sugar moiety, however, has not yet been positively identified. Formation of conjugate **II** was enhanced in the presence of CGA 185072 (Table I). Enhancement by a safener of glycosyl conjugation of a hydroxylated herbicide has been reported so far in one case by Lamoureux and Rusness [13]. These authors have demonstrated that the rate of O-glucosidation of hydroxylated chlorimuron ethyl in maize roots was accelerated in response to the safener BAS 145138.

In conclusion, the short-term metabolism studies presented here indicate that CGA 184927 is

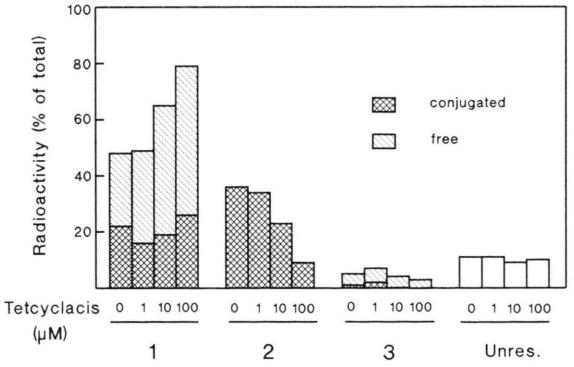

Fig. 4. Effect of tetcyclacis on the metabolism of CGA 184927 in wheat. Excised leaves were fed [^{14}C]CGA 184927 and tetcyclacis for 2 h and thereafter allowed to metabolize [^{14}C]CGA 184927 for 6 h. Total radioactivity extracted from the leaves was set 100%. See Fig. 2 for key to metabolites. Unres. = polar metabolites not resolved on TLC.

Table I. Effect of the safener, CGA 185072, on the conjugation of metabolite **2** in excised wheat leaves. [^{14}C]Labelled **2** was obtained from incubations of excised wheat leaves with [phenyl-U-^{14}C]CGA 184927 and purified by TLC and HPLC following cellulase cleavage. Excised wheat leaves were fed [^{14}C]labelled **2** for 2 h in the absence or presence of CGA 185072 and thereafter allowed to metabolize **2** for 1 h and 2 h prior to analysis by HPLC. Total extractable radioactivity was set 100%.

Metabolites	R_t [min][a]	Radioactivity (% of total) 1 h		2 h	
		−S[b]	+S	−S	+S
Metabolite **2**[c]	19.2	44.1	28.7	21.6	10.0
Conjugate **I**	16.2	20.8	22.1	25.1	25.9
Conjugate **II**	15.3	35.1	49.2	53.3	64.1

[a] Retention time on HPLC.
[b] Safener, CGA 185072.
[c] For structure of metabolite **2** see Fig. 2.

transformed in wheat and barley by similar pathways (Fig. 2). In these cereal crop species, CGA 184927 was readily deesterified to yield the herbicidally active acid. Further metabolism of the herbicide by pyridinyl-ring hydroxylation, ether cleavage, and glycosyl conjugation was shown to proceed at a comparatively slow rate in barley, which is more susceptible to CGA 184927 injury than wheat. Furthermore, it was shown that the safener, CGA 185072, accelerated herbicide metabolism in both wheat and, to a lesser degree, in barley. It can be concluded that the ability of CGA 185072 to protect wheat from CGA 184927, and to confer partial protection to barley, is related to the ability of the safener to stimulate herbicide metabolism in these cereal crops. In contrast, no further metabolism beyond ester hydrolysis of CGA 184927 and formation of glycosyl ester(s) was detectable in maize. Formation of glycosyl ester conjugates of aryloxyphenoxypropanoates in susceptible plants is generally considered not to be a stable detoxification step [7]. Moreover, there was no significant effect of CGA 185072 on herbicide metabolism in maize. This result explains the high susceptibility of maize to the herbicide, CGA 184927, both in the absence and presence of CGA 185072.

Acknowledgements

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