# Metabolism of the Herbicide 2-(2,4-Dichlorophenoxy)-propionic Acid (Dichlorprop) in Barley *(Hordeum vulgare)*

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*Dedicated to Professor Helmut Simon on the occasion of his 60th birthday*

Metabolism, Herbicide, Hydroxylation, Conjugation

The metabolism of the herbicide [$^{14}$C]2-(2,4-dichlorophenoxy)-propionic acid (dichlorprop) was studied in excised seedlings of barley *(Hordeum vulgare)*. It was rapidly taken up and metabolized by the plants yielding 5 metabolites. The metabolites, isolated by extraction with aqueous acetone, separated and purified by TLC, were identified by enzymatic, chemical, and spectrometric methods. Hydroxylation takes place at the 4-position of the aromatic ring, involving the NIH-shift of the chlorine to the 5-position and, to a minor extent, to the 3-position. The parent compound and also the 4-hydroxy derivatives undergo conjugation with mono- and diglucoses at the carboxyl and at the hydroxyl group, respectively. The time course of metabolism during 24, 48, and 72 h is presented, indicating interconversion reactions.

## Introduction

Although the auxin herbicide dichlorprop has been widely used in agriculture in the control of broad-leaved weeds for many years very little is known about its metabolism in higher plants and the possible contribution of this to its selective action. This strongly contrasts with the comprehensive understanding of the fate of the closely related herbicide 2,4-dichlorophenoxy-acetic acid (2,4 D) and other phenoxy acids which have been extensively studied in plants and plant cell cultures [3–7]. Conjugation with amino acids and sugars, ring hydroxylation, and side chain cleavage are the most important paths of phenoxy alkanoic acid metabolism.

In a short investigation in maple a slow decarboxylation of dichlorprop has been observed [8], and in a preliminary investigation with cereals regarding a residual analysis it has been found especially to be conjugated with sugars [9].

We report in this paper on the metabolism of dichlorprop in barley plants and especially on characterization of the different metabolites.

## Material and Methods

### *Radiochemicals and synthetic standards*

[1-$^{14}$C]Dichlorprop was synthesized from [1-$^{14}$C]2-bromo-propionic acid (Isocommerz GmbH, Dresden-Rossendorf) and 2,4-dichlorophenol in 5 N aqueous NaOH and converted to the potassium salt with methanolic $K_2CO_3$ [10]. Specific radioactivity was 55 MBq · mmol$^{-1}$.

4-Hydroxy-2,5-dichlorprop (F. 139–142 °C; $^1$H NMR: δ 1.7 (d, 3H, $-CH_3$); 4.7 (q, 1H, $-CH-$); 7.0 (s, 1H, aromatic proton at C-6); 7.1 (s, 1H, aromatic proton at C-3; MS (electron impact): *m/z* 250 ($M^+$, 53), 205 (12), 178 (100), 144 (29); IR (KBr): γ (CO) 1720 cm$^{-1}$) and 4-hydroxy-2,3-dichlorprop (F. 155–158 °C; $^1$H NMR: δ 1.69 (d, 3H, $-CH_3$); 4.71 (q, 1H, $-CH-$); 6.89 (s, 2H, aromatic protons); MS (electron impact): *e.g.* *m/z* 250 ($M^+$, 73), 206 (37), 178 (100), 144 (57); IR (KBr): γ (CO) 1720 cm$^{-1}$) were synthesized from ethyl α-bromopropionate or α-bromopropionic acid and 2,5-dichlorohydroquinone or 2,3-dichlorohydroquinone according to [11 and 12]. The corresponding acetyl and methyl derivatives were obtained by acetylation with pyridine/acetic anhydride and purification by

*Abbreviations:* dichlorprop, 2-(2,4-dichlorophenoxy)-propionic acid; TLC, thin layer chromatography; HPLC, high performance liquid chromatography; NMR, nuclear magnetic resonance; MS, mass spectrometry; Glc, β-D-glucosyl; LSC, liquid scintillation counting; TMS, tetramethylsilane; M, molecular ion peak; *m/z*, mass/charge; GOD–POD, glucose oxidase – peroxidase; ac, acetyl; ss, solvent system.

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preparative TLC in ss 1 (4-acO-2,5-dichlorprop, F. 123–125 °C, and 4-acO-2,3-dichlorprop) and methylation with diazomethane (4-meO-2,5-dichlorprop-me, F. 51–53 °C). The analytic data correspond with the suggested structures. Dichlorprop-glcac$_4$ was synthesized from dichlorprop and acetobromoglucose in the presence of triethylamine according to [13]. The compound shows the following data: $^1$H NMR: *e.g.* δ 1.67 (m, 3H, $-CH-C\underline{H}_3$); 1.9–2.1 (several s, 12H, 4× $-CO-CH_3$); 3.9–5.3 (4m, 6H at C-2 to C-6 of glc); 4.73 (m, 1H, $-C\underline{H}-CH_3$); 5.73 (m, 1H at C-1 of glc); 6.78 (d, 1H, aromatic proton at C-5); 7.13 (m, 1H, aromatic proton at C-6); 7.36 (d, 1H, aromatic proton at C-3); MS (electron impact): fragment with intact sugar-aglycon-bond: *m/z* 564 ($M^+$, 8); sugar fragments: *m/z* 331 (60), 289 (5), 271 (22), 242 (6), 211 (19), 169 (100), 145 (30), 139 (37), 127 (53), 115 (43), 109 (89); aglycon fragment: *m/z* 189 (43); MS (electron attachment): fragments with intact sugar aglycon bond: *e.g.* *m/z* 623 (1), 599 (19), 564 ($M^-$, 20), 528 (7), 505 (8), 401 (12); sugar fragments: *e.g.* *m/z* 347 (19), 289 (27), 141 (63), 127 (56); aglycon fragments: *e.g.* *m/z* 233 (61), 216 (20), 199 (53), 161 (100).

*Plant material and application*

Seedlings of barley (*Hordeum vulgare* cv. Vogelsanger Gold) were cultivated in soil in the greenhouse at 23–25 °C on a daily photoperiodic regime of 15 h light and 9 h darkness. In each experiment 10 g of 6-day-old excised seedlings were immersed for increasing time intervals (24, 48, and 72 h) in 10 ml aqueous solution containing 1 mg (0.37 mmol/$l^{-1}$) [1-$^{14}$C]dichlorprop (K-salt).

*Isolation of metabolites*

Following incubation, the excised plants were rinsed several times with water, cut into small pieces, homogenized with an Ultra-Turrax (Janke u. Kunkel KG, Staufen i. Br., FRG) in 50% aqueous acetone, and filtered. The solid material was shaken for 15 h with another portion of 50% aqueous acetone. The combined filtrates were evaporated to remove the acetone and the remaining aqueous phase was extracted successively with *n*-hexane and ethyl acetate.

Radioactivity of aliquots of all fractions was determined by LSC with a Tricarb 2660 (Packard Instruments, Chicago, USA). Before LSC the solid residue was combusted with a Micro-Mat BF 5010 sample oxidizer (Berthold-Frieseke GmbH, Karlsruhe, FRG). The metabolites from the different extracts were separated and purified by TLC. For quantification of metabolites the ratios of aglycones, determined after hydrolysis of an aliquot, were used, and the integrals of peak areas from radioscans were evaluated.

*TLC*

Preparative TLC was carried out on self-coated plates (silica gel 60 $PF_{254}$, Merck, 0.9 mm thick) with a 3 cm concentration zone (Kieselgur, Merck). For comparative TLC precoated silica gel plates ("Silufol $UV_{254}$", Kavalier, CSSR) were used. As solvent systems were used:

| | | |
|---|---|---|
| 1. toluene : dioxane : acetic acid | 90 : 25 : 2 | (v : v : v) |
| 2. benzene : acetone : acetic acid | 20 : 80 : 3 | (v : v : v) |
| 3. ethyl acetate : 2-propanol : water | 50 : 30 : 12 | (v : v : v) |
| 4. benzene : acetone : acetic acid | 80 : 16 : 4 | (v : v : v) |
| 5. 1-propanol : water | 64 : 36 | (v : v) |

The TLC plates were analyzed for radioactive zones with a Radioscanner II (Berthold, Wildbad, FRG).

*Hydrolysis and derivatization of conjugates*

Samples of conjugates (2–8 μg supposed glucose, calculated from radioactivity) were dissolved in 1 ml McIlvain buffer, pH 3.0, and incubated for 20 h at 37 °C with 250 μg dialyzed cellulase. Quantification of glucose was carried out by the glucose oxidase-peroxidase method [14]. For alkaline hydrolysis, an aliquot was treated with 1 ml 2.5% aqueous $NH_4OH$ (24 h, room temperature). Acid hydrolysis was carried out by treatment of an aliquot for 1 h with 1 ml 1 N HCl at 100 °C. Acetylation was done with pyridine : acetic anhydride 2 : 1 (v : v).

*Spectrometric methods*

$^1$H NMR spectra were recorded on a Bruker WP 200 spectrometer (Karlsruhe, FRG) at 200.13 MHz in $CDCl_3$. Chemical shift values are related to TMS.

Electron impact (2 to 4 eV) and electron attachment (10 to 16 eV) mass spectra were obtained with a "Manfred von Ardenne" mass spectrometer (Dresden, GDR).

The IR spectra were recorded by using a UR 20 spectrometer from VEB Carl-Zeiss, Jena, GDR.

## Results and Discussion

Application of dichlorprop as its water soluble potassium salt to the excised seedlings of barley resulted in high uptake. Table I shows the distribution of radioactivity in the obtained fractions and ratios of dichlorprop and its metabolites, both determined after different time intervals. In characteristic experiments the radioactivity inside the plants increased from 48% of applied radioactivity after 24 h (47% was extracted with aqueous acetone and 1% remained in the solid residue) to 83% (79% in the aqueous acetone extract and 4% in the solid residue) after 72 h. The radioactive metabolites were extracted from the aqueous phase with *n*-hexane and ethyl acetate. It was shown that the concentration of radioactivity, soluble in ethyl acetate, decreased with increasing incubation time while the water soluble amount of radioactivity increased, indicating transformation to polar metabolites. This was confirmed by TLC. Surprisingly the *n*-hexane extract, beside dichlorprop, contained considerable amounts of polar metabolites which may be adsorbed to lipophilic plant constituents. This was suggested, since these metabolites were no more soluble in *n*-hexane after TLC. Despite this quantitative differences in the metabolic pattern found after different incubation intervals, in all experiments the same metabolites were detected.

After purification and separation by TLC, chemical and enzymatical hydrolysis, derivatization, cochromatography with authentic reference compounds and quantitative determination of enzymatically released glucose were used for characterization of the metabolites.

Beside small portions of dichlorprop, 5 metabolites and a radioactive fraction of polar conjugates which was not mobile in the solvent systems used were found. In Table II the metabolites are listed according to decreasing TLC mobility, together with information on the $R_F$ values in the solvent systems used for separation and purification and approximate quantities formed in the 72 h experiment.

Peak 1 cochromatographed with dichlorprop (ss 4, $R_F$ 0.33; ss 1, $R_F$ 0.42). Peak 2 is present only in trace amounts and cochromatographed with 4-hydroxy-2,5-dichlorprop (ss 4, $R_F$ 0.26; ss 1, $R_F$ 0.32). Peak 3 ($R_F$ 0.6 in ss 2) was hydrolyzed under alkaline and acidic conditions to give dichlorprop. The acetylation product of peak 3 cochromatographed with the synthetic tetraacetylglucose ester of dichlorprop (ss 4, $R_F$ 0,40; ss 1, $R_F$ 0.5) suggesting that peak 3 is the monoglucose ester of dichlorprop.

Peak 4 was hydrolyzed under acidic conditions yielding an aglycone, which cochromatographed with 4-hydroxy-2,5-dichlorprop. The acetylation product of peak 4 is slightly more polar than that of peak 3 and can be extracted with aqueous sodium hydrogen carbonate from ethyl acetate, indicating a free carboxyl group. Therefore, we suggest that peak 4 is a glycoside of a hydroxylated dichlorprop, most probably a monoglycoside.

Peak 5 is the main metabolite of the hexane extract and is obtained from the ethyl acetate extract in nearly the same concentration as peak 3 and 4 together. It was hydrolyzed under acidic conditions to dichlorprop and the acetylation product cannot be extracted by sodium hydrogen carbonate. Comparative investigation and cochromatography with a

Table I. Distribution of radioactivity in the fractions obtained from excised barley seedlings treated with 1 mg [$^{14}$C]dichlorprop and ratios of dichlorprop and its metabolites, both determined after different time intervals.

| Fractions | Time intervals % of applied radioactivity 24 h | 48 h | 72 h |
|---|---|---|---|
| Solid residue | 1 | 3 | 4 |
| 50% aqueous acetone extract | 47 | 75 | 79 |
| *n*-Hexane | 14 | 29 | 37 |
| Ethyl acetate | 21 | 19 | 13 |
| Aqueous phase | 12 | 27 | 29 |
| Ratio dichlorprop : metabolites[a] | 33 : 67 | 22 : 78 | 11 : 89 |

[a] The ratios are related to the 50% aqueous acetone extract.

Table II. TLC data and approximate quantities of dichlorprop and its metabolites formed in excised barley seedlings.

| Peak Nr. | Metabolite[a] | TLC[b] $R_F$ | solvent system | approximate quantities[c] |
|---|---|---|---|---|
| 1 | dichlorprop | 0.4 | 1 | minor |
| 2 | 4-hydroxy-2,5-dichlorprop | 0.3 | 1 | trace |
| 3 | dichlorprop-glc | 0.6 | 2 | minor |
| 4 | 4-glc-O-2,5-dichlorprop | 0.5 | 2 | minor |
| 5 | dichlorprop-$glc_2$ | 0.5 | 3 | major |
| 6 | 4-$glc_2$-O-2,5-dichlorprop | 0.2 | 3 | major |
| 7 | polar conjugates[d] | 0 | 3 | minor |

[a] Metabolites are listed according to decreasing TLC mobility.
[b] For TLC conditions see "Materials and Methods".
[c] The approximate quantities were determined after 72 h (see also Table I). "Minor" means about 5–15% and "major" 25–50% of applied radioactivity.
[d] By hydrolysis dichlorprop, 4-hydroxy-2,5-dichlorprop and 4-hydroxy-2,3-dichlorprop were formed.

structurally elucidated metabolite of dichlorprop in plants and cell suspension cultures of tomato [15] have shown that peak 5 is identical with a disaccharide ester of dichlorprop, most probably containing two mols of glucose per mol dichlorprop.

From the aqueous phase a peak 6 could be separated (ss 3, $R_F$ 0.2) from more polar metabolites. This was hydrolyzed under acidic conditions and also enzymatically be cellulase yielding 4-hydroxy-2,5-dichlorprop. Following enzymatic hydrolysis, a molar ratio 4-hydroxy-2,5-dichlorprop:glucose of 1:2.43 (GOD–POD-test) was obtained. The acetylated peak 6 (ss 1, $R_F$ 0.3) could be extracted by sodium hydrogen carbonate from ethyl acetate. These results indicate that peak 6 is a glucoside of 4-hydroxy-2,5-dichlorprop with two mols of glucose.

Hydrolysis under acidic conditions of the mixture of most polar metabolites (peak 7) which could not be separated by TLC (*e.g.* ss 5) yields dichlorprop, 4-hydroxy-2,5-dichlorprop and 4-hydroxy-2,3-dichlorprop, identified by cochromatography with synthesized test substances and their acetylation products.

The results show that in barley seedlings hydroxylation takes place at the 4-position of the aromatic ring, involving the NIH-shift of the chlorine to the 5-position and, to a minor extent, to the 3-position. Ring hydroxylation and the NIH shift are common paths of metabolism of 2,4-D [16]. The 4-hydroxy derivatives were detectable in free form only in trace amounts (4-hydroxy-2,5-dichlorprop) or not at all (4-hydroxy-2,3-dichlorprop). Rather they underwent conjugation with carbohydrates at the hydroxyl group. The parent compound, dichlorprop, was conjugated to carbohydrates via the carboxyl group. Conjugating moieties in both cases were glucose and a diglucose. The diglucose conjugates of dichlorprop and 4-hydroxy-2,5-dichlorprop (dichlorprop-$glc_2$ and 4-$glc_2$-O-2,5-dichlorprop) were the major metabolites, and the glucose conjugates (dichlorprop-glc and 4-glc-O-2,5-dichlorprop) were minor ones. No conjugation with amino acids has been observed.

Conjugation with glucose as ester or glucoside is well known for 2,4-D and other phenoxy acids [17] too. However, intact disaccharide conjugates in the phenoxy acid series were identified until now only with 2-(2,4-dichlorophenoxy)-isobutyric acid [18].

By hydrolysis of the polar conjugate fraction, which was immobile in the solvent systems used, dichlorprop, 4-hydroxy-2,5-dichlorprop, and 4-hydroxy-2,3-dichlorprop were liberated. Side chain degradation was not detectable because of specific labelling of the carboxyl group. However, some loss in recovered radioactivity suggests liberation of $^{14}CO_2$.

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