Inhibitory Effects of Some Polysubstituted Phenoxyacetic Acid Derivatives on Photosynthetic Activity of Chloroplasts and on Chlorophyll Production in Wheat and *Chlorella vulgaris*

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The inhibition of oxygen evolution rate in spinach chloroplasts and chlorophyll production in algae *Chlorella vulgaris* as well as in wheat plants caused by some polysubstituted phenoxyacetic acid derivatives was investigated. 2,4,5-Trichlorophenoxyacetic acid and its derivatives exhibited substantially higher activity with respect to the inhibition of photosynthetic processes in spinach chloroplasts than the corresponding 2,4-dichloro and 2-methyl-4-chloro analogues. From the changes of EPR spectra of spinach chloroplasts in the presence of the studied compounds it can be supposed that these interact with both photosynthetic centres 1 and 2. The antialgal effect of the majority of studied compounds at the concentration 10^{-4} mol dm^{-3} is not pronounced.

2,4-Dichloro-, 2-methyl-4-chloro- and 2,4,5-trichlorophenoxyacetic acids belong to the auxin group of effectors with the pronounced plant growth-regulating activity. It is known that this activity depends remarkably on the concentration of the auxin-type effectors exhibiting stimulating effects at very low concentrations and inhibitory effects at high concentrations [1—3]. Recently a great variety of polysubstituted phenoxyacetic acid derivatives were synthesized and investigated from the viewpoint of diverse biological effects, *e.g.* antifungal, herbicidal and juvenile antihormones activity [4—10].

The aim of this paper was the investigation of inhibitory efficiency of some derivatives of 2,4-dichloro- (2,4-D), 2-methyl-4-chloro- (MCPA) and 2,4,5-trichlorophenoxyacetic acids (2,4,5-T) concerning photosynthetic processes in spinach chloroplasts and chlorophyll production in algae *Chlorella vulgaris* and in wheat.

EXPERIMENTAL

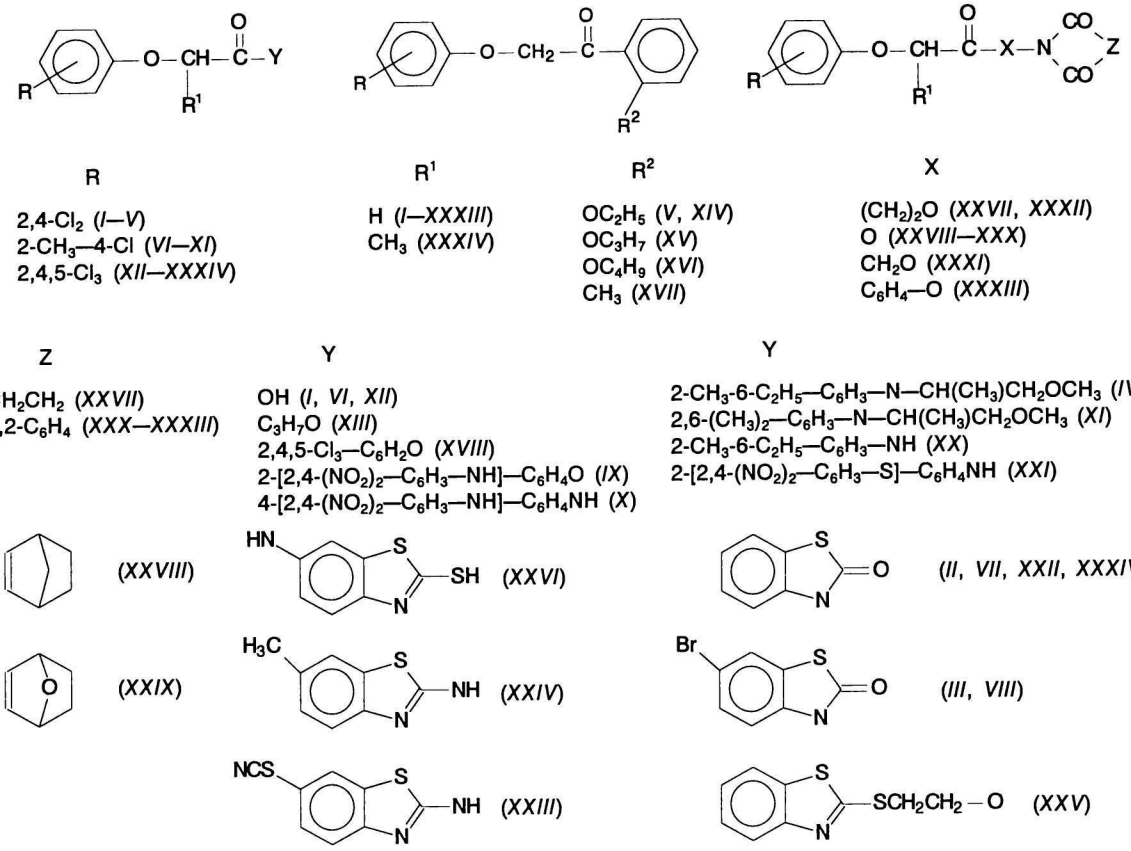
For the study of inhibitory effects of some polysubstituted phenoxyacetic acid derivatives on photosynthetic activity of chloroplasts and chlorophyll production in algae, 34 compounds were used (Scheme 1). The syntheses of studied effectors are summarized in the previous papers [4—9].

Spinach chloroplasts for studying of photosynthetic activity were prepared according to the procedure described in Ref. [11]. The oxygen evolution rate in spinach chloroplasts was determined spectrophotometrically (Specord UV VIS; Zeiss, Jena) using 2,6-dichlorophenolindophenol as electron acceptor at constant chlorophyll (Chl) concentration 30 μg cm^{-3} [12]. The inhibitory activity has been expressed by IC_{50} values, *i.e.* by concentration causing 50 % decrease of the activity of control sample. Because of low solubility of the studied compounds in water, these were dissolved in *N*,*N*-dimethylformamide (DMF) and the decrease of photosynthetic activity due to the presence of DMF was taken into account.

Chlorella vulgaris algae were stationary cultivated (7 d, 16 h light/8 h dark photoperiod; the details are in [13]) and the Chl content of algal suspension was determined spectrophotometrically after its extraction into *N*,*N*-dimethylformamide [14]. The samples contained a constant concentration of the studied compounds (10^{-4} mol dm^{-3}) dissolved in DMF. The resulting DMF concentration in the samples as well as in the control was adjusted to 1 %. (The presence of 1 % DMF in the samples itself causes a pronounced inhibition of Chl production in algae – approximately 55 % with respect to samples without DMF.)

The cultivation of wheat plants and the determination of Chl content was carried out according to [13]. The used concentrations of effectors were constant (10^{-4} and 10^{-5} mol dm^{-3}, respectively). The concentration of DMF used in the controls was the same as in the investigated samples.

EPR measurements were carried out with an instrument ERS 230 (WG, Akademie der Wissenschaften, Berlin) operating in X-band at 5 mW of microwave power. EPR spectra of untreated spinach chloroplasts as well as in the presence of studied compounds (0.05 mol dm^{-3}) were recorded in



Scheme 1

the dark and in the light. The content of Chl in the samples was 2 mg cm^{-3}. The irradiation was carried out with a 250 W halogen lamp through water filter.

RESULTS AND DISCUSSION

The effect of the studied compounds on oxygen evolution rate in spinach chloroplasts expressed by IC_{50} values is summarized in Table 1. The inhibitory activity of 2,4,5-T (*XII*) is by two orders higher than that of 2,4-D (*I*) and MCPA (*VI*), respectively. Stronger inhibition showed also the derivatives of 2,4,5-T (*XIV*, *XIX*, *XXII*) with respect to the corresponding 2,4-D (*IV*, *V*) and MCPA (*VII*) analogues. From the comparison of inhibitory activities of compounds *XIV—XVI* (2,4,5-T derivatives) it is evident that the increase of alkyl chain length of alkoxy substituent leads to a decreased inhibition. In contrast to this group, the increase of substituent lipophilicity can cause also an opposite effect (compounds *XX* and *IX*). This apparently contradictory behaviour can be explained as follows: for reaching the site of action the effector must cross hydrophilic as well as lipophilic regions of the thylakoid membrane in sufficiently high concentration. That means that too high lipophilicity of the molecule can strongly decrease solubility of the effector and so its penetration through the hydrophilic region of the membrane, which results in the subsequent decrease of inhibitory activity. On the other hand, however, the increase of lipophilicity can improve the ability of the effector to penetrate through the hydrophobic regions of the membrane. Thus the total lipophilicity of the molecule determined within the three investigated series by the structure of the substituent is important from the viewpoint of efficient transit of the effector through both the above-mentioned regions of the membrane.

The inhibitory activity of succinimido derivative *XXVII* is more than by one order lower than that of the corresponding phthalimido derivative *XXXII*. Higher inhibitory activity was shown also by the further investigated phthalimido derivative *XXX* with respect to its bicyclo analogues *XXVIII* and *XXIX*. The inhibitory activity of phthalimido derivatives *XXX—XXXII* decreases in the order *XXX*, *XXXII*, *XXXI*, similarly to the determined herbicidal activity of these compounds [7].

The study of the action of investigated compounds on spinach chloroplasts by EPR spectroscopy showed that the most effective derivatives — 2,4-D, 2,4,5-T, and *XXIX* — exhibit some changes in EPR spectra of chloroplasts in the $g = 2$ region (Fig. 1). Superposed EPR signals I ($g = 2.0026$) and II ($g = 2.0045$) illustrated in Fig. 1, belonging to the photo-

Table 1. IC_{50} Values for Oxygen Evolution Rate in Spinach Chloroplasts in the Presence of the Studied Compounds and Chlorophyll Production Inhibition by these Compounds in *Chlorella vulgaris* Algae at $c = 10^{-4}$ mol dm^{-3}

Compound	$IC_{50} \cdot 10^5$ / mol dm^{-3}	Inhibition / %
I	316.0	0
II	8.7	31.3
III	60.3	39.6
IV	40.7	5.7
V	15.8	–
VI	933.0	0
VII	81.3	45.3
VIII	26.9	46.1
IX	12.6	88.0
X	7.9	–
XI	38.0	3.1
XII	1.9	19.9
XIII	10.7	67.5
XIV	0.98	12.1
XV	3.4	0
XVI	3.4	–
XVII	1.5	13.7
XVIII	2.7	20.4
XIX	8.3	5.0
XX	52.6	15.6
XXI	2.5	–
XXII	8.1	15.5
XXIII	8.3	100
XXIV	3.4	19.7
XXV	19.1	53.2
XXVI	9.3	51.0
XXVII	39.8	12.3
XXVIII	3.7	65.0
XXIX	2.3	44.0
XXX	1.1	6.1
XXXI	24.0	–
XXXII	5.3	100
XXXIII	4.4	14.4
XXXIV	31.6	48.4

synthetic centres 1 and 2 (PS 1 and PS 2), respectively [15] show the ratio of signal intensity in the dark and in the light to be approximately 1.7 for untreated chloroplasts (Fig. 1, line *A*). The treatment of chloroplasts with the above-mentioned compounds affected both EPR signals I and II: in the dark their intensity was lowered, but the intensity of signal I showed an increase in the light (Fig. 1, lines *B* and *C*).

According to the present knowledge, the primary site of action of 2,4-D and 2,4,5-T herbicides with respect to photosynthesis inhibition remains still obscure. It is known that auxin-type herbicides cause morphological changes in plant chloroplasts, which are in many aspects similar to changes occurring during normal senescence [2]. From EPR spectra of chloroplasts treated with relatively high concentration of 2,4-D and 2,4,5-T it seems that these compounds interact with photosynthetic centres 1 and 2. Certain changes in EPR spectra of these photosystems in wheat and barley leaves treated with 2,4-D were obtained also by *Gribova et al.* [16]. The assumption that 2,4-D and 2,4,5-T derivatives affect the PS 1 and PS 2 is indirectly supported also by the results of *Glass* [17] who found that 2,4-D

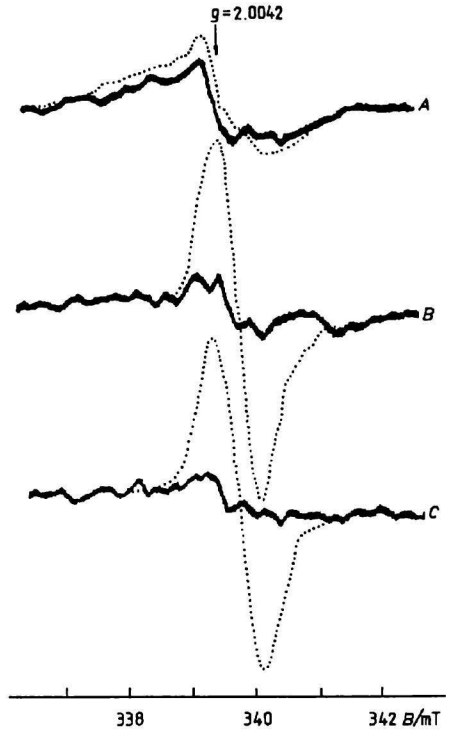


Fig. 1. EPR spectra of spinach chloroplasts: *A* – untreated; *B* – treated with 5×10^{-2} mol dm^{-3} 2,4-D; *C* – treated with 5×10^{-2} mol dm^{-3} 2,4,5-T in the dark (full line) and in the light (dotted line). The Chl concentration for all samples was 2 mg cm^{-3}.

and 2,4,5-T decreased the phase transition temperature of dipalmitoylphosphatidylcholine vesicles from gel to liquid crystalline phase. Similarly, if interactions between 2,4-D and 2,4,5-T with thylakoid membranes take place, the subsequent changes in the membrane structure can evoke also changes in PS 1 and PS 2 which are located in these membranes. The results obtained with EPR spectroscopy showed higher inhibitory efficiency of 2,4,5-T compared with 2,4-D — similarly to the results of the study of oxygen evolution rate in spinach chloroplasts (Table 1). Lower inhibitory activity determined with 2,4-D and 2,4,5-T derivatives with respect to the corresponding acids can be connected also with their limited solubility in investigated aqueous systems.

The inhibition of Chl production in green algae *Chlorella vulgaris* by the studied compounds showed significant differences among effects of investigated effectors at equimolar concentration $c = 10^{-4}$ mol dm^{-3} (Table 1). Total inhibition of Chl production caused only two derivatives of 2,4,5-T (*XXIII* and *XXXII*), further five compounds (*IX*, *XIII*, *XXV*, *XXVI*, *XXVIII*) inhibited Chl production in the range of 50—88 %. In the presence of 2,4-D and MCPA Chl production in algae was not affected, and the inhibitory effectiveness of 2,4,5-T was also relatively small (19.9 %) in contrast to its pronounced inhibitory effect

Table 2. Chlorophyll Production in Wheat Plants Cultivated in the Presence of the Studied Compounds at the Given Concentration with Respect to the Control

Compound	Chl production/%	
	$c/(10^{-4}$ mol dm$^{-3})$	$c/(10^{-5}$ mol dm$^{-3})$
VII	11.7 ± 0.5	83.1 ± 9.7
VIII	25.2 ± 4.1	75.9 ± 1.7
XII	14.7 ± 5.5	51.6 ± 6.8
XXII	49.4 ± 5.8	102.5 ± 5.4
XXXI	56.8 ± 9.0	103.0 ± 3.7

on oxygen evolution rate in spinach chloroplasts. It can be summarized that the majority of the investigated compounds do not belong to the group of effectors with pronounced antialgal activity.

The green mass as well as Chl production in plants is also affected by the studied compounds. The inhibitory effect of five investigated effectors with respect to Chl production in wheat is demonstrated in Table 2. The high inhibitory efficiency of 2,4,5-T (*XII*) partly decreases with its two substituted derivatives — *XXII* (benzothiazolinone derivative) and *XXXI* (phthalimido derivative), whereas the inhibition produced by compound *XXII* (2,4,5-T derivative) is lower than that of its MCPA analogue *VII*.

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WPAC/FECS - Definition of Analytical Chemistry Agreed at Euroanalysis VIII, 1993, Edinburgh (UK).

"Analytical chemistry is a scientific discipline which develops and applies methods, instruments and strategies to obtain information on the composition and nature of matter in space and time."

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