Inhibition of Oxygen Evolution Rate in Freshwater Algae *Chlorella vulgaris* by Some Anilides of Substituted Pyridine-4-carboxylic Acids

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The inhibitory activity of 11 anilides of 2-alkylsulfanylpyridine-4-carboxylic acids (R = SC_3H_7, SC_5H_{11}, $SCH_2C_6H_5$, S-iC_4H_9; substituents in the anilide moiety: 2′-OH, 4′-OH, 5′-Cl, 3′-Br, 4′-Br, 3′-CF_3, 5′-CF_3) on oxygen evolution rate (OER) in freshwater algae *Chlorella vulgaris* has been investigated. The IC_{50} values in the investigated set varied in the range from 47.7 μmol dm^{-3} (R = SC_3H_7, Y = X^2 = H, X^1 = 4′-Br) to 474.1 μmol dm^{-3} (R = $SCH_2C_6H_5$, Y = 2′-OH, X^1 = X^2 = H). The dependence of photosynthesis-inhibiting activity upon the lipophilicity of the substituents showed a quasi-parabolic course indicating that for the OER-inhibiting activity in *Chlorella vulgaris* the lipophilicity of the compound is determining.

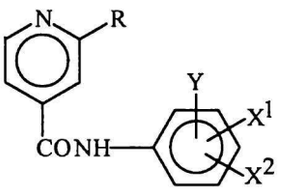
Acylanilides, benzanilides as well as anilides derived from heterocyclic compounds exhibit interesting biological effects, including photosynthesis-inhibiting activity [1—5]. For achievement of the required biological effect suitable lipophilicity of the inhibitor enabling the passage of the compound through the hydrophilic as well as hydrophobic regions of the thylakoid membranes is necessary. Due to formation of hydrogen bonds between CONH group of anilides and the target proteins in photosynthetic centres of thylakoid membranes changes in protein conformation may occur. Anilides belong to inhibitors of photosystem (PS) 2 and their mode of inhibition of PS 2 function is displacement of a plastoquinone (Q_B) from its binding site on the target protein [6]. This herbicide-binding protein is known to be one of the reaction-centre polypeptides of PS 2, called the D_1 polypeptide subunit [7].

This study is aimed to investigate the activity of some anilides of 2-alkylsulfanylpyridine-4-carboxylic acids concerning inhibition of oxygen evolution rate in green algae *Chlorella vulgaris* and to determine the correlation between the structure and the biological activity of the compounds.

EXPERIMENTAL

Studied anilides of 2-alkylsulfanylpyridine-4-carboxylic acids (ASPCA) (Formula 1) were prepared from corresponding acids by reaction of acyl chlorides with substituted anilines and aminophenols according to the methods described by *Miletín et al.* [8]. Their structure has been confirmed by elemental analysis, IR and NMR spectra. Anal. grade chemicals were employed for the preparation of all solutions.

The oxygen evolution rate (OER) in algal suspensions (*Chlorella vulgaris*) was measured at 24 °C by a Clark-type electrode (SOPS 31 atp., Chemoprojekt, Prague) in a chamber constructed according to *Bartoš et al.* [9]. Prior to the OER measurements the suspensions were accommodated in the dark (4 h). The samples were then illuminated with a 250 W halogen lamp from 0.3 m distance through a water filter. The composition of the algal cultivation medium was as follows: 20 mmol KNO_3, 2.5 mmol KH_2PO_4, 4.0 mmol $MgSO_4 \cdot 7H_2O$, 7.0 μmol $CaCl_2 \cdot 6H_2O$, 34.6 μmol $FeSO_4$, 34.6 μmol Na_2EDTA, 50.0 μmol H_3BO_3, 5.0 μmol $CuSO_4 \cdot 5H_2O$, 5.0 μmol $ZnSO_4 \cdot 5H_2O$, 5.0 μmol $MnCl_2 \cdot 4H_2O$, 1.5 μmol $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$, and 5.0 μmol $CoCl_2 \cdot 6H_2O$ in 1 dm^3 of H_2O; pH = 7.2. The chlorophyll mass concentration in the samples was 18 mg dm^{-3}. The concentration of chlorophyll was determined spectrophotometrically after its extraction into methanol according to *Wellburn* [10]. The activity of the studied anilides has been expressed by IC_{50} values, *i.e.* by molar concentrations causing a 50 % decrease of OER with respect to the untreated control. For low solubility of the studied compounds



R		Y		X^1		X^2	
$SCH_2C_6H_5$	(*I, V, VI, X*)	H	(*VI, X, XI*)	H	(*I, II*)	H	(*I—VIII*)
SC_5H_{11}	(*II, VII, IX, XI*)	2′-OH	(*I—V, VII*)	5′-Cl	(*III—V, VII*)	5′-Br	(*IX*)
SC_3H_7	(*III, VIII*)	4′-OH	(*IX*)	4′-Br	(*VI, VIII*)	5′-CF_3	(*X, XI*)
S-iC_4H_9	(*IV*)			3′-Br	(*IX*)		
				3′-CF_3	(*X, XI*)		

Formula 1

in water, these were dissolved in dimethyl sulfoxide. The applied solvent content (up to 4 vol. %) practically did not affect OER in the algal suspension of *Chlorella vulgaris*.

The values of logarithms of partition coefficients (log P) computed using a program ACD/Log P ver. 1.0 (Advanced Chemistry Development Inc., Toronto) were as follows: 3.75 (*I*), 4.24 (*II*), 4.55 (*III*), 4.90 (*IV*), 5.13 (*V*), 5.28 (*VI*), 5.62 (*VII*), 5.77 (*VIII*), 6.66 (*IX*), 7.01 (*X*), 7.50 (*XI*).

RESULTS AND DISCUSSION

The studied anilides of 2-alkylsulfanylpyridine-4-carboxylic acids (Formula 1) inhibited photosynthetic electron transport in green algae *Chlorella vulgaris*, which was reflected in the inhibition of oxygen evolution rate. The inhibitory activity expressed by IC_{50} values varied in the investigated set in the range from 47.7 μmol dm^{-3} (R = SC_3H_7, Y = X^2 = H, X^1 = 4′-Br) to 474.1 μmol dm^{-3} (R = $SCH_2C_6H_5$, Y = 2′-OH, X^1 = X^2 = H). The dependence of logarithms of IC_{50} values on the lipophilicity of the compounds expressed by log P showed a quasi-parabolic course indicating that for the biological activity the lipophilicity of the compound is determining (Fig. 1). The determined values of log $\{1/IC_{50}\}$ used for the evaluation of structure—activity relationships were as follows: 3.3205 (*I*), 3.4801 (*II*), 3.6718 (*III*), 3.9020 (*IV*), 4.0531 (*V*), 4.2541 (*VI*), 4.1500 (*VII*), 4.3215 (*VIII*), 4.2801 (*IX*), 4.0550 (*X*), 4.0106 (*XI*) and the correlation between the inhibitory activity and the lipophilicity of anilides of 2-alkylsulfanylpyridine-4-carboxylic acids can be expressed by the following correlation equation

$$\log\{1/IC_{50}\} = -2.386\,(\pm 0.789) + 2.134\,(\pm 0.285)\log P - 0.171\,(\pm 0.025)(\log P)^2$$

$$r = 0.960 \quad s = 0.103 \quad F = 47.37 \quad n = 11$$

Our previous study showed that the investigated

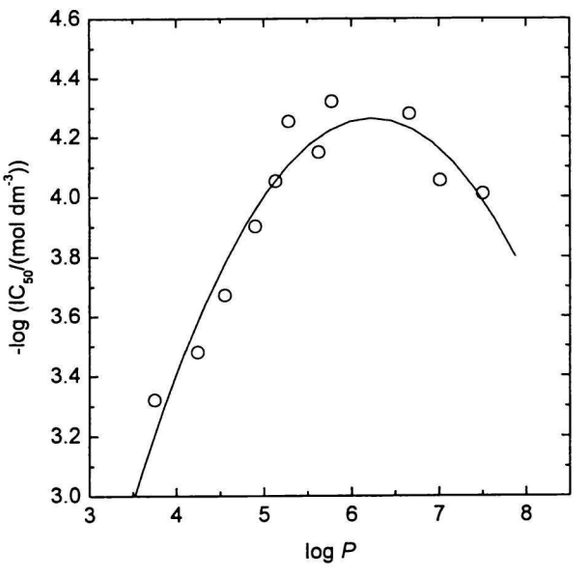


Fig. 1. The dependence of inhibition of OER in *Chlorella vulgaris* on the lipophilicity of the studied anilides of 2-alkylsulfanylpyridine-4-carboxylic acids.

anilides of 2-alkylsulfanylpyridine-4-carboxylic acids inhibited also the photosynthetic electron transport in spinach chloroplasts and the corresponding IC_{50} values varied in the range from 4.8 μmol dm^{-3} to 69.1 μmol dm^{-3} (compounds *VII* and *XI*) and the lipophilicity of the most active compounds was about log P = 5.0—5.5 [11]. Using EPR spectroscopy the site of action of the anilides of 2-alkylsulfanylpyridine-4-carboxylic acids in the apparatus of spinach chloroplasts was studied [11]. We found that these anilides interact predominantly with the intermediate $D^+(Tyr_D)$ and in a pronouncedly less extent also with the intermediate Z^+ (Tyr_Z). Tyr_D and Tyr_Z are tyrosine radicals situated on the donor side of PS 2 at the 161st position in D_2 and D_1 proteins [12]. The intensive interaction of the studied anilides with Tyr_D which is situated in less polar environment of the thylakoid membranes can be connected

with the presence of hydrophobic alkylsulfanyl substituent in their molecules. Similar site of action was determined for anilides of 2-alkylpyridine-4-carboxylic acids [4] as well as for a set of benzanilides and thiobenzanilides [2, 3]. The previous results of an experiment with the artificial electron donor (diphenylcarbazide) acting in Z^+/D^+ intermediate showed that in spinach chloroplasts also some member of the photosynthetic electron transport chain between Z^+/D^+ and plastoquinone was partially damaged by 2-alkylsulfanylpyridine-4-carboxylic acids [10]. With respect to similar effects produced by anilides of 2-alkylsulfanylpyridine-4-carboxylic acids on inhibition of oxygen evolution rate in spinach chloroplasts and in *Chlorella vulgaris* it is probable that also the site of action of these inhibitors in both photosynthesizing organisms will be the same. Thus, we assume that similarly to the interaction of these anilides with the target redox-active tyrosines Tyr_D and Tyr_Z situated in D_2 and D_1 proteins on the donor side of photosystem 2 of spinach chloroplasts such interaction will occur also in green algae *Chlorella vulgaris*.

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