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Inhibitory effects of some esters of 2- and 3-substituted alkoxyphenylcarbamic acids on photosynthetic characteristics

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Abstract

The inhibitory effect of 23 *N*-alkyl-4-piperidylesters (alkyl = ethyl-butyl) (APEA) and 8 *N*-ethyl-2-pyrrolidinylmethylesters (EPMEA) of 2- and 3-substituted alkoxyphenylcarbamic acids (alkoxy = butoxy-heptyloxy-) on photosynthetic Hill reaction activity of spinach chloroplasts and on chlorophyll (Chl) synthesis in green algae *Chlorella vulgaris* was investigated. Inhibitory activities of these compounds were strongly connected with the lipophilicity of the whole molecule. A lower inhibitory activity of 2-alkoxy-substituted derivatives in relation to the corresponding 3-substituted ones was confirmed. Electron spin resonance (ESR) spectra of spinach chloroplasts demonstrated that the studied compounds affected the structure of photosystem (PS) 2 with the release of Mn^{2+} ions into interior of thylakoid membranes.

Introduction

Esters of substituted alkoxyphenylcarbamic acids exhibit several interesting biological properties which are strongly connected with their amphiphilic structure. To their most important biological activities belong antimicrobial (Cižmárik *et al.* 1987), antiarythmic (Bachratá *et al.* 1987, Csöllei *et al.* 1987) and local anaesthetic effects (Csöllei 1981, Csöllei *et al.*1986). Since such effects are caused by interactions of these compounds with biological membranes (Seemann 1972), further effects based on similar principles can be expected including the inhibition of photosynthetic processes (Mitterhauszerová *et al.* 1991). The aim of this work was to investigate the effect of 23 *N*-alkyl-4-piperidylesters (alkyl = ethyl-butyl) and 8 *N*-ethyl-2-pyrrolidinylmethylesters of 2- and 3-substituted alkoxyphenylcarbamic acids (alkoxy = butoxy-heptyloxy) on some characteristics of photosynthesis.

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Material and methods

N-alkyl-4-piperidylesters (APEA) and *N*-ethyl-2-pyrrolidinylmethylesters (EPMEA) of 2- and 3-substituted alkoxyphenylcarbamic acids were synthesized according to Csöllei (1981) and Csöllei *et al.* (1986).

The effect of these compounds on Chl content of stationary cultivated algae *Chlorella vulgaris* (7-d old, 16/8 h photoperiod) was investigated using the methods described by Mitterhauszerová *et al.* (1991). Chl was extracted into *N,N*-dimethylformamide and determined spectrophotometrically (*Specord UV VIS,* Zeiss, Jena, Germany) according to Inskeep and Bloom (1985). For the studied compounds the values of IC_{50} and MIC (concentrations causing a 50% decrease and total inhibition of Chl synthesis in algae, respectively) were determined.

Spinach chloroplasts were prepared by a partly modified procedure of current preparation methods described by Walker (1980) using TRIS buffer (20 mM, pH 7.0) containing 0.4 M saccharose and 0.2 mM $MgCl_2$ (for details see Šeršeň *et al.* 1990).

Hill reaction activity in spinach chloroplasts was determined spectrophotometrically (*Specord UV VIS,* Zeiss, Jena, Germany) as the rate of oxygen evolution at constant Chl concentration (30 g m^{-3}) using 2,6-dichlorophenolindophenol as an electron acceptor (Buschmann and Grumbach 1985). The phosphate buffer used for these measurements (20 mM, pH = 7.2) contained saccharose (0.4 M), $MgCl_2$ (5 mM) and NaCl (15 mM) and the samples were irradiated from the distance of 10 cm with halogen lamp (250 W) using water filter to prevent overheating of samples. For the compounds studied, the values of IC_{50}, *i. e.* the concentrations at which the oxygen evolution rate of chloroplasts drops to 50 % with respect to the corresponding value of untreated ones, were determined.

ESR measurements were carried out with an instrument *ERS 230* (WG, Akad. Wissensch., Berlin, Germany) operating in X-band at 5 mW of microwave power. ESR spectra of untreated spinach chloroplasts and in the presence of studied compounds (0.05 M) were recorded in the dark and in the light. Chl content in the samples was 4 kg m^{-3}. The samples were irradiated with a 250 W halogen lamp through a water filter.

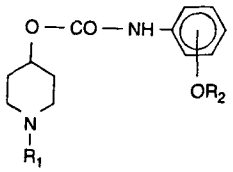
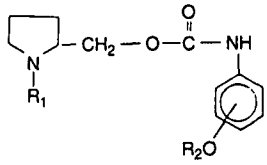
Results and discussion

The studied APEAs and EPMEAs inhibited both the Hill reaction in spinach chloroplasts and the Chl synthesis in *C. vulgaris*. The extent of these inhibitory activities expressed by IC_{50} and MIC values depended upon the number of carbon atoms in the alkoxysubstituent of molecule and its position on the benzene ring (Fig.1, Table 1).

The comparison of EPMEAs and APEAs with N-alkyl = ethyl confirmed a lower inhibitory efficiency of the N-ethyl-2-pyrrolidinylmethylesteric group in the molecule with respect to the N-ethyl-4-piperidylesteric group (Fig. 1A and 1B, Table 1); the inhibitory efficiency of all 2-substituted derivatives was significantly lower than that of the 3-substituted ones.

The increase of the inhibitory effects with the prolongation of the alkyl chain of the alkoxy-substituent can be explained by an increase in lipophilicity of these substances. The increased lipophilicity of the molecule enables its better penetration through hydrophobic regions of the thylakoid membrane to the site of action. Similarly, the lipophilicity of the studied APEAs could be increased also by prolongation of the *N*-alkyl substituent on esteric group from ethyl to propyl or butyl, respectively, but in these cases the raise of the inhibitory activity with the alkyl chain

Table 1. Algicidal effect of APEA and EPMEA (expressed by minimum inhibitory concentration (MIC) with respect to chlorophyll synthesis in *Chlorella vulgaris*

		MIC [10^{-5} mol m^{-3}]			
					
		APEA		EPMEA	
R_1	R_2	2-subst.	3-subst.	2-subst.	3-subst.
C_2H_5	C_4H_9	5.00	1.10	6.62	3.98
	C_5H_{11}	5.00	0.91	6.03	2.51
	C_6H_{13}	3.31	0.60	5.01	1.00
	C_7H_{15}	1.02	1.15	1.58	1.10
C_3H_7	C_4H_9	6.00	1.62	-	-
	C_5H_{11}	2.51	1.00	-	-
	C_6H_{13}	2.51	0.87	-	-
	C_7H_{15}	2.51	1.00	-	-
C_4H_9	C_4H_9	2.00	1.58	-	-
	C_5H_{11}	2.00	-	-	-
	C_6H_{13}	3.98	1.00	-	-
	C_7H_{15}	3.55	1.51	-	-

length of the alkoxysubstituent was less pronounced (Fig. 1C and 1D with respect to 1A).

As mentioned above, the biological activity of the given effector is connected with the transport to its site of action. This can be secured only by a certain lipophilicity or hydrophilicity of the molecule which enables to cross hydrophobic as well as hydrophilic regions of biological membranes (Balaz *et al.* 1988). It means that with the prolongation of the *N*-alkyl substituent in the esteric group of APEAs, the hydrophobicity of the whole molecule increases and so the ability of derivatives with longer alkoxy-substituent (hexyloxy- and heptyloxy-) to cross hydrophilic regions of thylakoid membranes can be partly limited.

In all studied series the inhibitory activities of the 3-substituted alkoxy-derivatives were stronger as these of the 2-substituted ones. This can be explained with a higher distance between the alkoxy-substituent and the carbamate group of molecule, when

mutual intramolecular interactions are strongly lowered or practically eliminated. In the case of 2-substituted derivatives the distortion of the plane of the benzene ring against the plane of the carbamate group takes place what leads to perturbation of molecular planarity and is manifested as a secondary steric effect (Čižmárik *et al.* 1987).

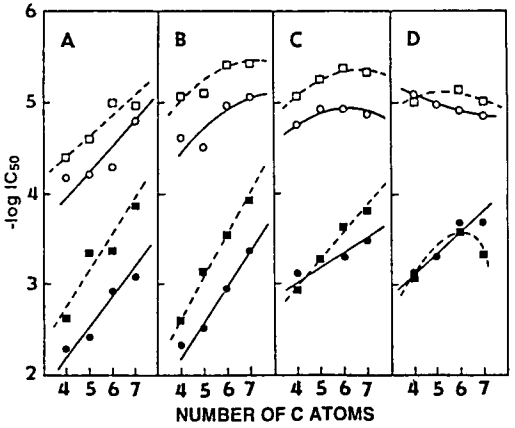


Fig.1. Dependence of inhibition of chlorophyll synthesis in *Chlorella* (*empty symbols*) and Hill reaction (*full symbols*) in spinach chloroplasts (expressed as -log IC_{50} values) on the number of carbon atoms in the alkoxysubstituent of EPMEA (A) and APEA with N-alkyl = ethyl (B), propyl (C) and butyl (D); 2-(*circles*) and 3-alkoxy-substituted (*squares*) derivatives.

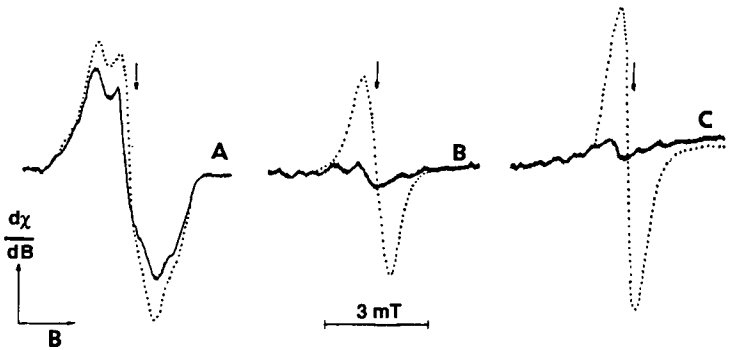


Fig.2. ESR spectra of untreated spinach chloroplasts (lines A) and chloroplasts treated with 0.05 M N-ethyl-2-pyrrolidinylmethylesters of 2-butoxyphenylcarbamic acid (B) and 2- heptyloxyphenylcarbamic acid (C). The dotted lines in B and C were recorded at 0.5 amplification. The arrows correspond to the *g* factor value of 2.0026.

The studied compounds affected also ESR spectra of spinach chloroplasts. The ESR spectrum of untreated chloroplasts consists of two superimposed signals I and II (see Fig.2, line A) which belong to photosynthetic centres PS 1 and PS 2, respectively (Hoff 1979, 1987). These signals increse after switching on light *ca.* 1.7-fold. In the presence of the studied compounds the intensity of ESR signal II decreased, but the intensity of ESR signal I at irradiation increased (Fig. 2, lines B and C). The raise of the signal I in the light can be explained by destruction of PS 2 causing partial

decrease or total disappearance of electron transfer to PS 1. The intensity of the signal I in the light is proportional to inhibitory efficiency of the effector (compare Fig.2, lines B and C with corresponding MIC values in Table 1 and IC_{50} values in Fig.1).

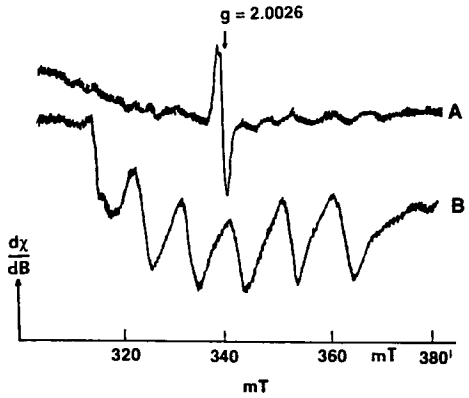


Fig.3. ESR spectra of Mn^{2+} ions in untreated chloroplasts (line A) and in chloroplasts treated with 0.05 M *N*-ethyl-2- pyrrolidinylmethylester of 2-alkoxyphenylcarbamic acid (line B).

The assumption concerning destruction of PS 2 by the studied compounds was supported also by the detection of Mn^{2+} ions in the ESR spectra (Fig. 3). Manganese ions are bonded to 33 kDa protein which is located on the donor side of PS 2 (Govindjee and Wasielewski 1989) and due to destruction of PS 2 their release from the protein complex into the interior of thylakoid membranes can be observed.

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