

Inhibitory Effect of Piperidinoethylesters of Alkoxyphenylcarbamic Acids on Photosynthesis

K. KRÁĽOVÁ¹, F. ŠERŠEŇ¹ and J. ČIŽMÁRIK²

¹ Faculty of Sciences, J. A. Comenius University,
842 15 Bratislava, Czechoslovakia

² Faculty of Pharmacy, J. A. Comenius University,
832 32 Bratislava, Czechoslovakia

Abstract. Piperidinoethylesters of 2-,3- and 4-alkoxysubstituted phenylcarbamic acids (alkoxy = methoxy – decyloxy) inhibit photosynthetic processes in algae and plant chloroplasts. The inhibitory activity is strongly dependent on the alkyl chain length of the alkoxy-substituent showing a typical quasi-parabolic dependence with maximum effect at 6 – 8 carbon atoms in the alkyl chain. The alkoxy-substitution in position 2 decreases the inhibitory activity of a compound when compared with its 3- and 4-substituted analogues. ESR studies of spinach chloroplasts confirm that the compounds studied cause destruction of PS II whereby, in the presence of the most effective of the derivatives tested, Mn^{2+} ions are released from the protein complex.

Key words: Piperidinoethylesters of alkoxyphenylcarbamic acids — *Chlorella vulgaris* — Spinach chloroplasts — Photosynthesis inhibition — ESR spectroscopy

Introduction

Piperidinoethylesters of alkoxyphenylcarbamic acids (PAA) are amphiphilic compounds with several biological properties connected with their effects on biological membranes: antimicrobial activity (Mlynarčík and Čižmárik 1976); antiarrhythmic activity (Kozlovský et al. 1976, 1982); local anaesthetic activity (Čižmárik et al. 1976). PAA interact with significant components of cell membranes - lipids and proteins - causing a perturbation of the membranes thus influencing biological functions. The measure of the above mentioned inhibitory effects of PAA is strongly dependent on the alkyl chain length of the alkoxy-substituent, partly also on its position on the benzene ring of the molecule. Due to amphiphilic structure of PAA, in aqueous solutions associates similar to those of surfactants can be formed (Pešák et al. 1977, 1980; Kopecký et al. 1978).

This paper presents the results of a study dealing with effects of piperidi-

noethylesters of 2-, 3- and 4- alkoxyphenylcarbamic acids upon growth and chlorophyll synthesis in the alga *Chlorella vulgaris* and with their effects upon photosynthesis in plant chloroplasts.

Materials and Methods

The synthesis of PAA has been described by Čižmárik and Borovanský (1975). In the present study hydrochlorides of 2-, 3- and 4- substituted PAA derivatives with alkoxy = methoxy - decyloxy were used.

Green algae *Chlorella vulgaris* were stationary cultured and their growth and chlorophyll content were determined according to Mitterhauszerová et al. (1991). Minimum algicidal concentration (MAC), i.e. minimum PAA concentration causing total inhibition of chlorophyll synthesis in algae, and PAA concentration causing its 50 % inhibition (IC_{50}) were determined.

The spinach chloroplasts were prepared using the method described by Šeršeň et al. (1990). The rate of oxygen evolution in spinach chloroplasts was determined spectrophotometrically (Specord UV VIS, Zeiss Jena, Germany) by classical Hill reaction at constant chlorophyll concentration (30 $\mu\text{g/ml}$) using phosphate buffer (20 mmol/l; pH 7.2) containing sucrose (0.4 mol/l), MgCl_2 (5 mmol/l) and NaCl (15 mmol/l) and 2,6-dichlorophenolindophenol as an electron acceptor. The samples were illuminated, from a distance of 10 cm, with a halogen lamp (250 W) using water filter to eliminate warming of the samples.

ESR measurements were carried out with an X - band operating instrument ERS 230 (ZWG, AdW Berlin, Germany). The ESR method used for the determination of photosynthesis inhibition in plant chloroplasts in the presence of amphiphilic compounds has been described in a previous paper (Šeršeň et al. 1990).

Results and Discussion

The interactions of photosynthesis inhibitors with photosynthetic centers PS I and PS II can be detected using ESR spectra (Gribova et al. 1985; Hoff 1979, 1987). In systems containing plant chloroplasts treated with the amphiphilic molecules studied (PAAs), signal II in ESR spectra decreases or totally disappears, whereas signal I on the light shows a great increase. This can be seen with ESR spectra obtained in the presence of piperidinoethylester of 2-heptyloxyphenylcarbamic acid which is one of the most effective compounds of the PAA series studied (Fig. 1, line B). In addition, the most effective derivatives release Mn^{2+} ions from protein complex into the thylakoid membrane interior. In these cases 6 lines of fine structure appear in ESR spectra indicating released Mn^{2+} ions (Fig. 2, line B; the superimposed signals I and II can be seen between lines 3 and 4 of the Mn^{2+} spectrum). These results confirm that the site of the inhibitory action of the studied PAAs on the photosynthetic apparatus of spinach chloroplasts is photosystem PS II.

Fig.3 shows the dependence of log MAC for chlorophyll synthesis in *Chlorella*

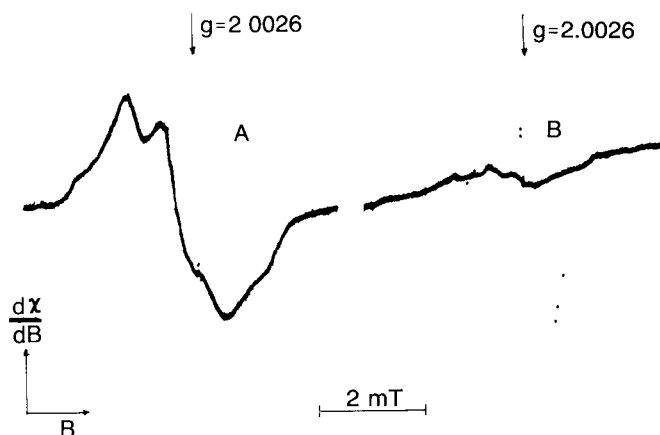


Figure 1. ESR spectra of spinach chloroplasts containing 4 mg chlorophyll/ml. *A*: untreated chloroplasts; *B*: chloroplasts treated with piperidinoethylester of 2-heptyloxyphenylcarbamic acid (0.1 mol/l). Full line, spectra recorded in the dark; dashed line, corresponding spectra at illumination. The dashed line in spectrum *B* was recorded at 0.5 amplification.

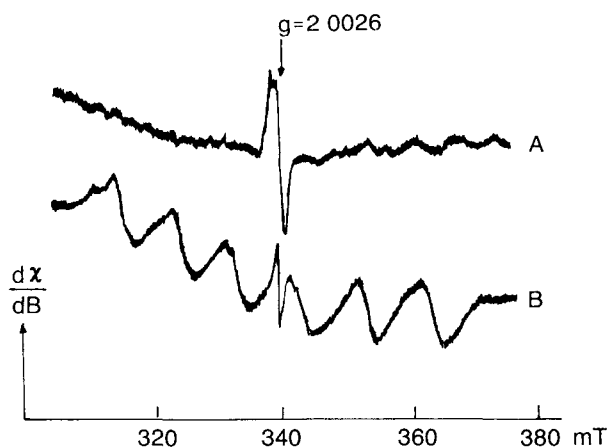


Figure 2. ESR spectrum of Mn^{2+} ions in spinach chloroplasts. *A*: intact chloroplasts; *B*: chloroplasts treated with 0.05 mol/l piperidinoethylester of 2-heptyloxyphenylcarbamic acid.

vulgaris on the alkyl chain length for 2-,3- and 4-alkoxy-substituted derivatives of PAAs. The Figure shows similar dependences of minimum bactericidal concentra-

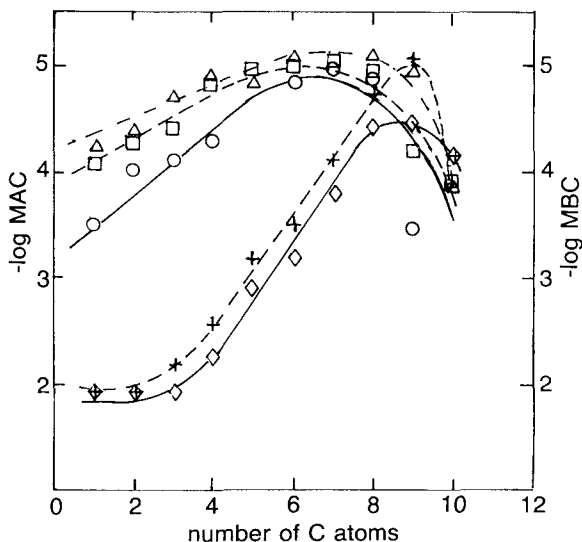


Figure 3. The dependence of logarithms of minimum algicidal concentration (MAC) and minimum bactericidal concentration (MBC) towards *Staphylococcus aureus* on the number of carbon atoms in PAA alkoxy-substituent. MAC: 2-(○), 3-(□) and 4-substituted (△) derivatives; and MBC: 2-(◇) and 3- or 4- substituted (+) derivatives. The corresponding values of MBC were taken from Čižmárik et al. (1987).

tions (MBC) for *Staphylococcus aureus* as determined by Čižmárik et al. (1987). From Fig. 3 it is obvious that the inhibitory effect of PAAs in both cases is dependent on the number of carbon atoms in the respective PAA alkoxy-substituent, showing maximum activity at 6 to 8 (MAC) or 9 (MBC) carbon derivatives, respectively; the activity of 2-substituted derivatives is generally lower than that of their 3- or 4-substituted analogues. Derivatives with shorter chains of the alkoxy-substituent (methyl – heptyl) have algicidal activity at significantly lower PAA concentrations than required for bactericidal effect on *Staphylococcus aureus*. This high inhibitory efficiency of PAA in relation to photosynthesizing organisms (algae) can be connected with effective destruction of the photosynthesizing apparatus by the amphiphilic molecules. The MAC value for the most effective derivative (octyl) is $7.9 \mu\text{mol/l}$, i.e. 3.3 mg/l .

The decrease of the rate of oxygen evolution in spinach chloroplasts observed in the presence of PAA (expressed as the corresponding IC_{50} values) is also strongly dependent on the alkyl chain length of the alkoxy-substituent (Fig.4). The most effective compounds in each homologous series were octyl-derivatives; similar to previous results, the inhibitory activities of 3- and 4-substituted derivatives were in general stronger than those of 2-substituted derivatives.

The effectiveness of photosynthesis inhibition in algae and plant chloroplasts, which depends strongly on the alkyl chain length of the PAA alkoxy-substituent, shows a typical quasi-parabolic dependence characteristic for several biological effects of amphiphilic compounds (Devínský et al.1985, 1990). The dependence of MAC on the PAA partition coefficient (*n*-octanol – phosphate buffer of pH 7.3)

Figure 4. The dependence of the rate of oxygen evolution in spinach chloroplasts (expressed in terms of $\log IC_{50}$ values) upon the alkyl chain length of PAA alkoxy-substituent. (For symbols see Fig. 3, $\log MAC$ values).

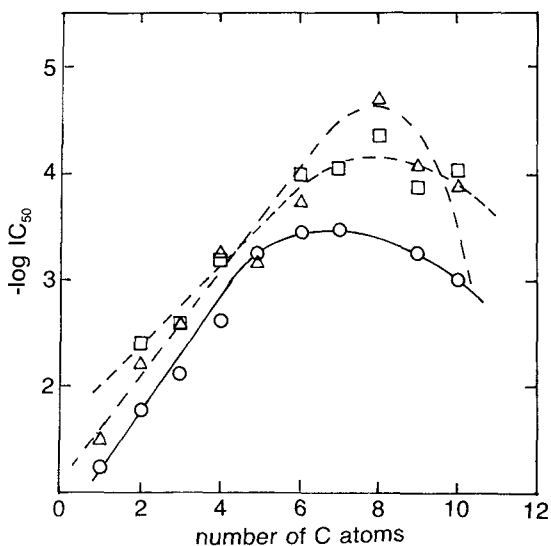
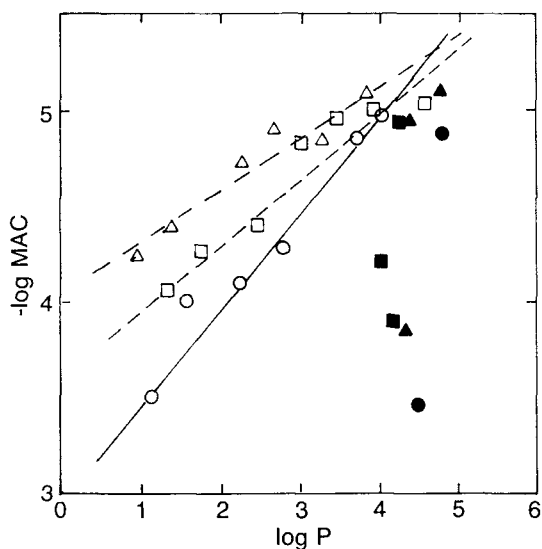


Figure 5. The dependence of $-\log MAC$ on the logarithm of partition coefficient P (for symbols see legend to Fig. 3; filled symbols correspond to 8 - 10 - C derivatives; the corresponding P values determined experimentally for systems *n*-octanol - phosphate buffer of pH 7.3 were taken from Pešák et al. (1980)).



is linear only for the first 7 members of all the 3 series studied (methyl-heptyl), with the corresponding correlation coefficients ranging between 0.96 - 0.98 (see Fig. 5). This supports the important role of hydrophobic interactions between PAAs and the lipid parts of membranes in influencing biological function. On the other hand, for derivatives with longer alkyl chain (octyl-decyl) not only the bio-

logical activity but also the partition coefficient showed a certain decrease with the increasing number of C atoms in the alkoxy-substituent (see Fig. 3, and values of partition coefficient in Pešák et al. (1980)). This decrease of partition coefficient can be explained by self-association of PAAs with longer alkyl chains in aqueous solution (Pešák et al. 1980). Thus, the algicidal effect is caused by individual PAA molecules, a decreased concentration of the monomers resulting in a lowered inhibitory activity.

Another possible explanation for the above observations might be also the so-called "cut - off" effect which has been discussed in detail by Balgavý et al. (1984), Devínsky et. al. (1990) and Gallová et al. (1990), or its combination with the self-association effect.

Generally, the inhibitory activity of 2-substituted PAA derivatives upon photosynthesis was weaker than that of their 3- or 4-substituted analogues. Framework molecular models of the studied PAAs show that 2-substituted derivatives having the alkoxy-substituent in close vicinity to carbamate bond can provoke a secondary sterical effect, distortion of the benzene ring plane against that of the carbamate group (Čižmárik et al. 1976). This leads to perturbation of molecule planarity and is reflected also in conjugation of π bonds of benzene ring through NH up to CO group in the molecule, thus changing electron density (charge) on the carbonyl group, which is one of the possible binding sites of carbamate acid derivatives on receptors or reaction centers of membranes (Čižmárik and Borovanský 1975). Probably, this secondary sterical effect is the cause of the weaker inhibitory efficiency of 2-substituted derivatives. In the case of 4-substituted derivatives, interactions between alkoxy-substituent and carbamate group are practically eliminated, and the inhibitory activity is stronger.

Summarizing it can be concluded that PAAs interact with thylakoid membranes of photosynthesizing organisms causing destruction of PS II with subsequent release of Mn^{2+} ions. The weakest inhibitory effect determined with 2-substituted PAA derivatives is probably connected with the sterical effect.

References

- Balgavý P., Gawrisch K., Frischleder H. (1984): Effect of N- alkyl-N, N, N- trimethylammonium ions on phosphatidylcholine model membrane structure as studied by ^{31}P -NMR. *Biochim. Biophys. Acta* **772**, 58—64
- Čižmárik J., Borovanský A. (1975): Synthesis, u.v. and i.r. spectra of 2-piperidinoethyl esters of alkoxyphenylcarbamic acids. *Chem. Papers* **29**, 119—123
- Čižmárik J., Borovanský A., Švec P. (1976): Study of local anaesthetics LII. Piperidinoethyl esters of alkoxyphenylcarbamic acids. *Acta Fac. Pharm. Univ. Comen.* **29**, 53—80
- Čižmárik J., Trupl J., Pešák M. (1987): A correlation between antimicrobial activity to *Staphylococcus aureus* and of selected physico-chemical parameters in the series of

- hydrochlorides of piperidinoethylesters of alkoxy-substituted phenyl-carbamic acids. *Českoslov. Farm.* **36**, 345—348 (in Slovak)
- Devínský F., Lacko I., Mlynarčík D., Račanský V., Krasnec Ľ. (1985): Relationship between critical micelle concentrations and minimum inhibitory concentrations for some non-aromatic quaternary ammonium salts and amine oxides. *Tenside Deterg.* **22**, 10—15
- Devínský F., Kopecká-Leitmanová A., Šeršeň F., Balgavý P. (1990): Cut-off effect in antimicrobial activity and in membrane perturbation efficiency of the homologous series of *N,N*-dimethylalkylamine oxides. *J. Pharm. Pharmacol.* **42**, 790—794
- Gallová J., Devínský F., Balgavý P. (1990): Interaction of surfactants with model and biological membranes. II. Effect of *N*-alkyl-*N,N,N*-trimethylammonium ions on phosphatidylcholine bilayers as studied by spin probe ESR. *Chem. Phys. Lipids* **53**, 231—241
- Gribova Z. P., Zinchenko V. A., Gunar L. E. (1985): Changes in ESR spectra of wheat and barley leaves under many years treatment of plantations by 2,4-D. *Izv. Akad. Nauk SSSR, Ser. Biol. No. 1*, 72—80 (in Russian)
- Hoff A. J. (1979): Application of ESR spectroscopy in photosynthesis. *Phys. Rep.* **54**, 75—200
- Hoff A. J. (1987): Electron paramagnetic resonance in photosynthesis. In: *Photosynthesis* (Ed. J. Ames), pp. 97—123, Elsevier Science Publishers Amsterdam
- Kopecký F., Záhorka V., Vojteková M., Čižmárik J. (1978): Cryoscopic properties of aqueous solutions of heptacaine and some its analogues. *Chem. Papers* **32**, 42—50
- Kozlovský J., Čižmárik J., Pešák M., Borovanský A. (1976): Studies of local anaesthetics LVIII. Comparison of the anti-arrhythmic effectiveness of heptacaine and lidocaine. *Českoslov. Farm.* **25**, 218—221 (in Slovak)
- Kozlovský J., Čižmárik J., Pešák M., Inczinger F., Borovanský A. (1982): Antiarrhythmic activity of heptacaine and some of its derivatives. *Arzneim.-Forsch.-Drug Res.* **32**, 1032—1036
- Mitterhauszerová Ľ., Kráľová K., Šeršeň F., Blanáriková V., Csöllei J. (1991): Effects of substituted aryloxyaminopropanols on photosynthesis and photosynthesizing organisms. *Gen. Physiol. Biophys.* **10**, 309—319
- Mlynarčík D., Čižmárik J. (1976): Antimicrobial efficiency of ω -piperidinoethyl esters of *n*-alkoxyphenylcarbamic acids. *Folia Microbiol.* **21**, 75—76
- Pešák M., Stankovičová M., Lukáš A., Borovanský A., Kopáčová Z. (1977): Studies of local anaesthetics LXI. Physicochemical properties of basic esters of 4-alkoxy-3-methyl-phenylcarbamic acids. *Českoslov. Farm.* **26**, 104—107 (in Czech)
- Pešák M., Kopecký F., Čižmárik J., Borovanský A. (1980): Study of local anaesthetics. Part 70. Some physico-chemical properties of piperidinoethylesters of alkoxyphenyl-carbamic acids. *Pharmazie* **35**, 150—152
- Šeršeň F., Balgavý P., Devínský F. (1990): Electron spin resonance study of chloroplast photosynthetic activity in the presence of amphiphilic amines. *Gen. Physiol. Biophys.* **9**, 625—633