Dimethylaminoethyl Alkoxyphenylcarbamates as Photosynthesis Inhibitors

[a]K. KRÁĽOVÁ, [a]F. ŠERŠEŇ, and [b]J. ČIŽMÁRIK

[a]*Institute of Chemistry, Faculty of Natural Sciences, Comenius University, CS-842 15 Bratislava*

[b]*Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Comenius University, CS-832 32 Bratislava*

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Dimethylaminoethyl 2-, 3-, and 4-alkoxyphenylcarbamates (n-alkoxy = methoxy to decyloxy) inhibit photosynthetic processes in algae and plant chloroplasts. The inhibitory activity of studied compounds shows a significant increase with the prolongation of the alkyl chain of the substituent and is suppressed only at derivatives with longer (C_8—C_{10}) alkoxy substituent. The inhibitory activity of 2-substituted derivatives is lower than that of their 3- and 4-substituted analogues. The most effective compounds exhibit algicidal effects at concentration 6 x 10^{-6} mol dm^{-3}.

Essential photosynthetic processes are concentrated in thylakoid membranes of cells of photosynthesizing organisms [1]. Amphiphilic molecules of the carbamate type are able to interact with free and membrane-bound proteins as well as with hydrophobic lipidic parts of membrane, which results in the change of the biological function of membrane with respect to the untreated one [2—5]. Such molecules can be incorporated into membrane causing expansion of the bilayer and changing electrical properties and fluidity of the membrane [3—6]. Amphiphilic amines affect the structure of photosystem II (PS II) and the electron transfer to photosystem I (PS I). Their inhibitory effects on photosynthesis correlate with their potency to perturb the lipid membrane structure [3, 7].

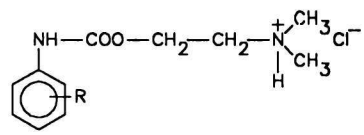
The aim of our study was to examine the effect of dimethylaminoethyl esters of 2-, 3-, and 4-alkoxy-substituted phenylcarbamic acids on photosynthesis inhibition in plant chloroplasts and *Chlorella vulgaris* algae.

EXPERIMENTAL

The synthesis of dimethylaminoethyl 2-, 3-, and 4-alkoxyphenylcarbamates (DEPA) is described in [8], some of their physicochemical and biological properties are reported in [9—11]. All of the studied compounds were used in the form of chlorides (Formula 1).

The cultivation of algae *Chlorella vulgaris* and the determination of growth and synthesized chlorophyll content in algae is in detail described in our previous paper [7].

Spinach chloroplasts were prepared according to *Šeršeň et al.* [3]. The rate of oxygen evolution by Hill reaction in spinach chloroplasts was determined spectrophotometrically on a Specord UV VIS apparatus (Zeiss, Jena) using 2,6-dichlorophenolindophenol as an electron acceptor. The applied phosphate buffer (c = 0.02 mol dm^{-3}) adjusted to pH = 7.3 contained also sucrose (c = 0.4 mol dm^{-3}),



$R = OC_nH_{2n+1}$ $n = 1—10$

Formula 1

$MgCl_2$ (c = 5 mmol dm^{-3}), and NaCl (c = 15 mmol dm^{-3}). The concentration of chlorophyll in the samples was constant (ρ = 30 μg cm^{-3}) and samples were illuminated from the distance of 10 cm with a halogen lamp (250 W) using water filter to exclude the warming of samples.

RESULTS AND DISCUSSION

Inhibitory activity of three homologous series of dimethylaminoethyl 2-, 3-, and 4-alkoxyphenylcarbamates on photosynthesis in plant chloroplasts and in green algae *Chlorella vulgaris* was investigated. Fig. 1 shows the dependence of their algicidal efficiency expressed by means of log MIC values (MIC is the minimum concentration of DEPA causing total inhibition of chlorophyll synthesis in algae) on the number of carbon atoms in alkoxy substituent of the compounds. From these dependences it is evident that the algicidal effect is strongly dependent on the alkyl chain length

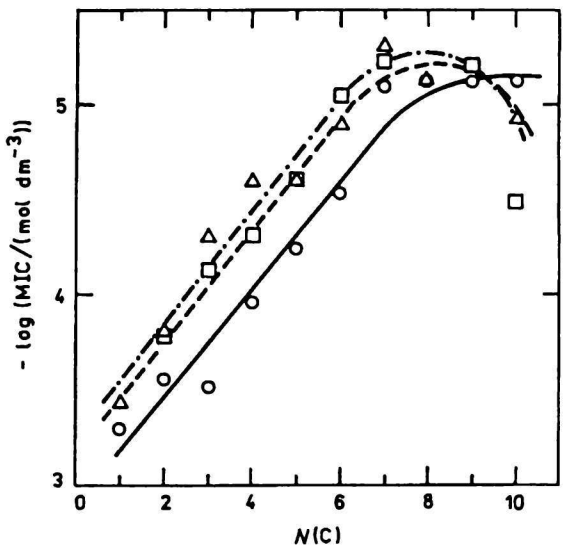


Fig. 1. Dependence of minimum algicidal concentration (MIC) on the number of carbon atoms in DEPA alkoxy substituent; 2- (○), 3- (□), and 4-substituted (△) derivatives.

of the alkoxy substituent and partly also on the position of the alkoxy substitution on the benzene ring of molecule. The algicidal activity of 2-substituted derivatives is lower than that of their 3- and 4-substituted analogues. The dependences illustrated in Fig. 1 are practically linear only for the first seven members of the investigated series (corresponding correlation coefficients are 0.96 for 2-, 0.99 for 3-, and 0.98 for 4-substituted derivatives); with the further prolongation of the alkyl chain no changes or a certain decrease of inhibitory activity can be observed.

Very similar results have been obtained also by the investigation of the rate of oxygen evolution by Hill reaction in plant chloroplasts (Fig. 2), which showed a marked decrease, *i.e.* an increased inhibitory activity (expressed by means of inhibitory concentration values IC_{50} corresponding to the DEPA concentration causing 50 % inhibition with respect to control samples) with the prolongation of the alkoxy substituent. The behaviour of derivatives with longer alkyl chain (C_8—C_{10}) showing minimum changes with its further prolongation was also in good agreement with the above-mentioned results obtained with algae.

The linear increase of inhibitory potency with the increase of number of C atoms in the alkoxy substituent can be explained by amphiphilic properties of DEPA. Based on these properties it can be assumed that predominantly take place interactions of DEPA molecules with lipids of thylakoid membranes. It is generally known that the partition coefficient between lipid and water in a homologous series of amphiphilic compounds having an alkyl chain in their molecule increases exponentially with the alkyl chain prolongation [12]. In the paper of *Bachratá et al.* [9] the values of DEPA partition coefficients are presented for the system n-octanol—water and they despite of certain deviations (mainly for 3-substituted series) exhibit the above-mentioned behaviour.

The dominant role of hydrophobic interactions between DEPA and thylakoid membrane can be confirmed using suitable correlations of biological efficiency with a parameter characterizing lipophilicity of the given molecule. For these

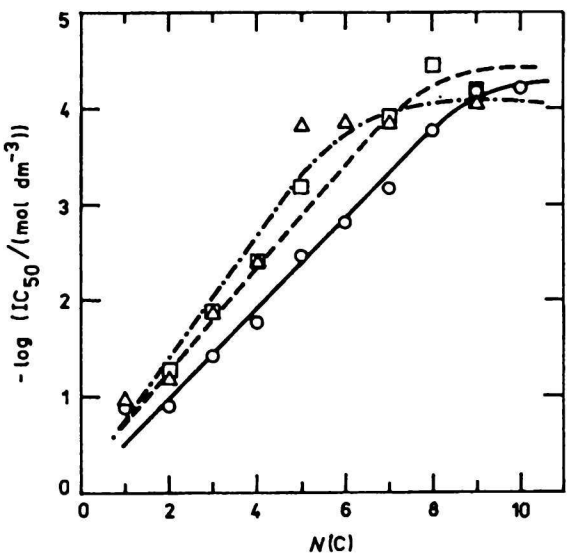


Fig. 2. Dependence of the rate of oxygen evolution in spinach chloroplasts by Hill reaction (expressed by IC_{50} values) upon the alkyl chain length of DEPA alkoxy substituent (symbols as in Fig. 1).

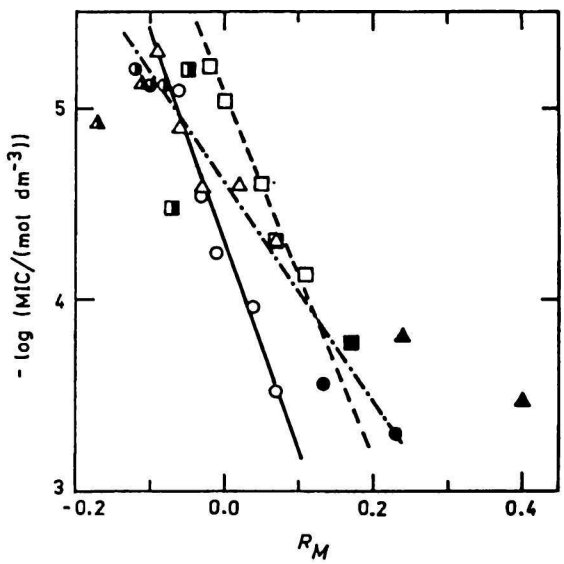


Fig. 3. Dependence of – log MIC on the R_M values of DEPA (symbols as in Fig. 1; full symbols correspond to C_1 and C_2, empty symbols to C_3—C_7 and half-full symbols to C_8—C_{10} derivatives). R_M values obtained from partition chromatography are taken from [9].

correlations we have used R_M values obtained from partition chromatography instead of usually employed classic partition coefficients because of their relative oscillations, mainly for 3-substituted series [9]. As shown in [9], the relation of R_M values to the number of carbon atoms in the alkoxy substituent is linear with the exception of the first two members (methoxy and ethoxy) of the homologous series. It was found that not only methyl and ethyl derivatives, but also derivatives with longer C_8-C_{10} alkyl chain exhibit a similar deviation from the linear dependence of – log MIC *vs.* R_M (Fig. 3). Derivatives with the octyloxy—decyloxy substituent have exhibited also deviations from the linear course of dependences illustrated in Figs. 1 and 2. This typical "cut-off" behaviour can be explained with the compartment theory [13] according to which long-chain homologues exhibit less ability to penetrate through hydrophilic compartments (aqueous regions) in contrast to short-chain homologues which have limited ability to cross the hydrophobic compartments (lipidic bilayers). It means that just molecules with medium chain length possess the optimal properties for transport to their site of action and show maximal biological activity.

The lower inhibitory activity of 2-alkoxy-substituted derivatives with respect to their 3- and 4-alkoxy-substituted analogues can be explained by secondary steric effect which is induced due to mutual interactions between alkoxy substituent and the carbamate group. This effect is in detail described in [14].

EPR investigations concerning photosynthesis inhibition in plant chloroplasts with amphiphilic amines structurally similar to those used in the present study have shown that such molecules inactivate photosynthetic centre PS II causing interruption of electron flow to photosynthetic centre PS I. This assumption has been confirmed also by the detected release of Mn^{2+} ions from the complex with protein (M_r = 33 000) situated on the donor side of PS II into interior of thylakoid membrane [3].

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