Photosynthesis Inhibition Produced by 2-Alkylthio-6-R-benzothiazoles

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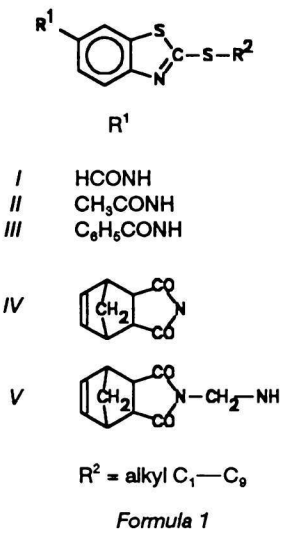
2-Alkylthio-6-R-benzothiazoles inhibit photosynthetic processes in plant chloroplasts and algae *Chlorella vulgaris*. The inhibitory effect is strongly dependent on the alkyl chain length of the compound, as well as on the nature of its substituent in position 6, whereby the total inhibitory effect is determined by the lipophilicity of the given molecule.

Substituted derivatives of benzothiazole show several interesting biological properties, for instance antimicrobial [1—3], antimycobacterial [4—7], or plant growth-regulating activity [1, 2]. For individual derivatives of the homologous series of 2-alkylthio-6-aminobenzothiazoles their activities against anaerobic sporulating bacteria [8], yeast-like microorganisms [9], as well as against mycobacteria [10] have been determined. Interesting results were obtained with 6-amino-2-mercaptobenzothiazole derivatives showing stimulating effect on the growth of model animals — chickens [11].

Studies of relationships between the chemical structure and biological activity have shown that many herbicides acting as photosynthesis inhibitors possess in their molecules an $\geq$N—C(=X)— group (X = O or N, not S) and a hydrophobic residue in close vicinity to the first group [12, 13]. *Shipman* [14, 15] concluded that the hydrophilic part of a herbicide binds electrostatically to the terminus of an α-helix at a highly charged amino acid, whereby the hydrophobic part of the inhibitors extends into the hydrophobic part of the membrane.

This paper is aimed to investigate the effect of some 2-alkylthio-6-R-benzothiazoles with R = formamido (*I*) (n-alkyl = C_2—C_9), acetamido (*II*) (C_2, C_4—C_6, C_8, C_9), benzoylamino (*III*) (C_1—C_4, C_6—C_9), bicyclo[2.2.1]hept-5-ene-2,3-dicarboximido (*IV*) (C_1—C_9), and bicyclo[2.2.1]hept-5-ene-2,3-dicarboximidomethylamino (*V*) (C_1—C_5, C_7) group on photosynthetic processes in *Chlorella vulgaris* and plant chloroplasts.



R^2 = alkyl C_1—C_9

Formula 1

EXPERIMENTAL

The synthesis of the studied compounds is described in [4, 5, 7, 16, 17].

The effect of these compounds on chlorophyll synthesis in stationary-cultivated *Chlorella vulgaris* algae was investigated according to *Mitterhauszerová et al.* [18] at the constant compound concentration 1 x 10^{-4} mol dm^{-3}. Because of low solubility of the studied compounds in water, these were dissolved in *N,N*-dimethylformamide (DMF) and the resulting DMF content in the samples as well as in the control was adjusted to 1 vol. % (the presence of 1 vol. % DMF in the samples itself causes a pronounced inhibition of chlorophyll synthesis in algae — approximately 55 % with respect to samples without DMF).

Spinach chloroplasts applied for the study of photosynthetic activity were prepared as described in [19] by a partly modified procedure of current preparation methods summarized in [20]. The rate of oxygen evolution in spinach chloroplasts and its influencing by the presence of the studied compounds was determined spectrophotometrically (Specord UV—VIS, Zeiss, Jena) by Hill reaction at constant chlorophyll concentration

(30 μg cm^{-3}) using 2,6-dichlorophenolindophenol as an electron acceptor [21]. The phosphate buffer used for these measurements (c = 20 mmol dm^{-3}; pH = 7.3) contained saccharose (c = 0.4 mol dm^{-3}), $MgCl_2$ (c = 5 mmol dm^{-3}), and NaCl (c = 15 mmol dm^{-3}) and the samples were illuminated from the distance of 10 cm with halogen lamp (250 W) using water filter to exclude the warming of samples. The activity of the studied compounds concerning photosynthesis inhibition in plant chloroplasts was expressed by inhibitory concentration values IC_{50} corresponding to concentrations causing 50 % inhibition with respect to the control. The decrease of photosynthetic activity due to the presence of DMF was taken into account (the highest used DMF content — 5 vol. % — caused approximately 25 % decrease of the oxygen evolution rate with respect to samples without DMF).

RESULTS AND DISCUSSION

The dependences of the negative logarithm of IC_{50} values on the alkyl chain length of the molecule for five investigated homologous series are illustrated in Fig. 1. A quasi-parabolic course of these dependences is typical for all studied series, similarly to that which was observed with series *I* and *II* by investigating their antimycobacterial

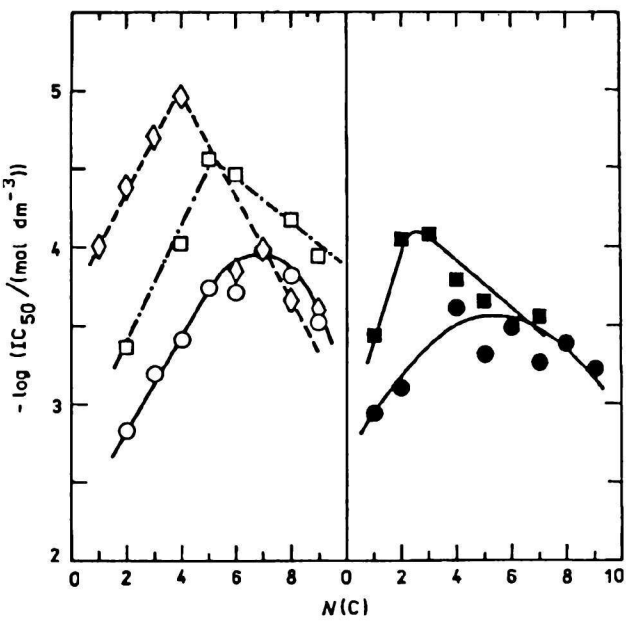


Fig. 1. Dependence of the negative logarithm of IC_{50} values concerning the oxygen evolution rate in spinach chloroplasts by Hill reaction on the number of carbons of the alkyl chain of 2-alkylthio-6-R-benzothiazoles (R = formamido (○), acetamido (□), benzoylamino (◇), bicyclo[2.2.1]hept-5-ene-2,3-dicarboximido (●), and bicyclo[2.2.1]hept-5-ene-2,3-dicarboximidomethylamino (■) group.

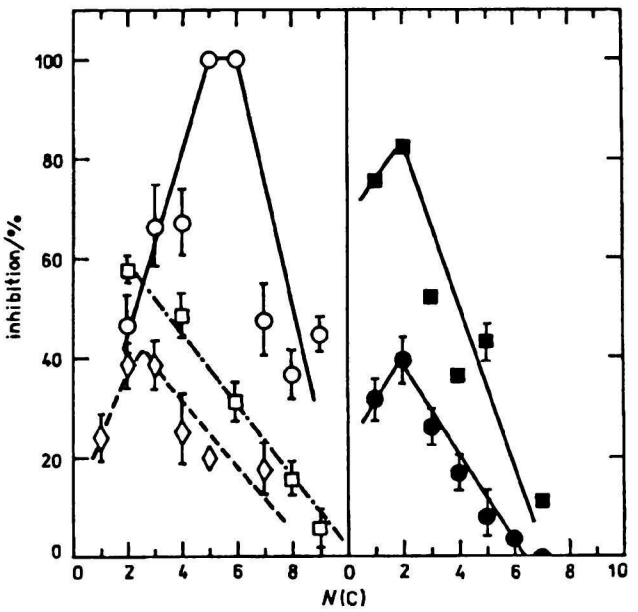


Fig. 2. Dependence of chlorophyll synthesis inhibition in algae *Chlorella vulgaris* in the presence of 6-alkylthio-6-R-benzothiazoles (c = 1 x 10^{-4} mol dm^{-3}) on their alkyl chain length (symbols as in Fig. 1).

activity against typical tuberculosis mycobacteria *M. tuberculosis* $H_{37}R_v$ as well as against the atypical strain *M. kansasii* [5, 7]. The effect of equimolar concentration (1 x 10^{-4} mol dm^{-3}) of the studied compounds on chlorophyll synthesis in *Chlorella vulgaris* (Fig. 2) shows also a pronounced parabolic dependence on the alkyl chain length of the molecule of the studied homologous series.

Many biological activities of long-chain substances show often this so-called "cut-off" effect — a decreased activity for the more lipophilic substances within the homologous series [5, 7, 22]. It is known that molecules with an alkyl chain can interact with biological membrane constituents, mainly lipids, causing perturbation of the membrane with subsequent changes of its biological function. The biologically active compounds, in order to reach their site of action, must penetrate through several compartments of membrane (*e.g.* series of lipid bilayers separated by aqueous layers). It means that the optimal effect is shown by molecules with suitable lipophilicity, which enables to cross both the above-mentioned compartments [23]. The dependence of the negative logarithm of IC_{50} values on the alkyl chain length of the compound, determined in the system of plant chloroplasts with partially damaged membranes, showed a significant role of the substituent in position 6 with respect to the studied inhibitory activity. The increasing of its lipophilicity in comparable series (Fig. 1, compounds *I*—*III* or *IV*, *V*) leads to higher activity of compounds having

shorter alkyl chains, with subsequent strong decrease of this activity with the further prolongation of the alkyl chain. The drop of the activity at derivatives with longer alkyl chains is the most pronounced in series having the most lipophilic substituents in position 6 (Fig. 2). With these series (*III* and *V*) the maximum of activity is shown by derivatives with shorter alkyl chains in comparison to corresponding series with more hydrophilic substituents in position 6 (C_4 in the series *III*, C_5 in *II* and C_8 in *I*; C_2 in *IV* and C_4 in *V*).

In contradiction to the effects exhibited by the studied compounds on the rate of oxygen evolution in spinach chloroplasts, the decrease of chlorophyll synthesis in algae *Chlorella vulgaris* in the case of series *I*—*III* was more strongly influenced by the presence of compounds with lower lipophilicity of the substituent in position 6 (the inhibitory efficiency decreases in the order 6-formamido, 6-acetamido, 6-benzoylamino derivatives). It can be then assumed that higher lipophilicity of the substituent in position 6 at equal chain length of the alkyl substituents diminishes the possibility of the compounds to penetrate through the intact outer algal cell membrane, resulting in decreased inhibitory activity of the compounds. This assumption is supported by lower antimycobacterial activities of 6-benzoylamino derivatives with respect to their 6-formamido or 6-acetamido analogues [4, 5, 7]. On the other hand, all bicyclo[2.2.1]-hept-5-ene-2,3-dicarboximidomethylamino derivatives showed higher photosynthesis inhibition in plant chloroplasts and in *Chlorella vulgaris* algae as well than the corresponding bicyclo[2.2.1]hept-5-ene-2,3-dicarboximido derivatives (see Figs. 1 and 2).

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