Bleaching Activity of New 2-Phenylpyridazinones: Structure-Activity Relationship

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New 2-phenylpyridazinones, analogs of 4-chloro-5-methylamino-2-phenylpyridazin-3(2H)ones, were assayed for their phytotoxic activity to interfere with plant pigment formation. Six derivatives with $-SCF_3$, $-OCF_2CHFCF_3$, $-OCHF_2$, $-CN$, or $-Br$ in *meta* position of the phenyl ring exhibited similar or better bleaching activity than the *m*-CF_3 analog (norflurazon). The activity is predominantly determined by positive σ values and by lipophilicity of the substituent. On the basis of 11 analogs, a quantitative structure-activity relationship by a Hansch equation was accomplished with σ_m and the lipophilicity parameter π as variables.

Introduction

Substituted phenylpyridazinones can act as "bleaching" herbicides, *i.e.*, they interfere with carotenoid biosynthesis and prevent the subsequent build-up of chlorophyll and other constituents of the thylakoid [1]. This phytotoxic effect depends on various substituents [2–4, see the latter for review]. In a recent publication, bleaching activity of such compounds was quantitatively assayed by measuring their interference with the greening process, using a sensitive *Scenedesmus* mutant [3]. As pointed out previously [5], this algal model system allows for fast and reliable data on structure-activity relationships. We found that the prominent effect of the substituents is by influencing the charge distribution of the phenylpyridazinone molecule. Pigment bleaching improved firstly by substituents with increasing σ_m values at position 4, secondly with decreasing σ_p values at position 5 of the pyridazinone ring, and thirdly by substituents with increasing σ_m or σ_p values at the meta position of the phenyl moiety ([3]; see formula in Table).

Consequently, the synthesis of 4-chloro-5-methylamino-2-phenylpyridazin-3(2H)ones with substituents of high positive σ_m or σ_p values in meta position at the phenyl ring was proposed [3] to obtain 2-phenylpyridazinones with stronger bleaching activity than available so far. In the present investigation, we report the bleaching activity of such newly synthesized pyridazinones and discuss the influence of substituents at the phenyl ring of the pyridazinone molecule resulting in more or less effective bleaching compounds.

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Materials and Methods

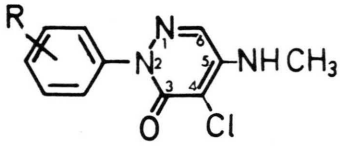
The I_{50} values for pigment bleaching were determined by interference of the compounds with the greening process of the WDG mutant of the green alga, *Scenedesmus acutus*, as described [5]. The chlorophyll content of the cells after 6 days of greening was determined in the presence of 3 to 4 concentrations of pyridazinones in the range of the presumed I_{50} value and the I_{50} values obtained by a Dixon plot. Growth of the cells, measurement of chlorophyll and packed cell volume were performed according to [3]. Occasionally, total carotenoids were determined; their level changed like that of chlorophyll.

The phenylpyridazinones were dissolved in either methanol (No. 1–5, 8–10, 12, 14 of the Table), dimethylformamide (Nos. 11, 13), or 50% dimethylformamide plus 50% methanol (Nos. 6, 7). Optical measurement of 4-chloro-5-methylamino-2-(3-nitrophenyl)-pyridazin-3(2H)one (No. 11) was carried out in a Shimadzu double-beam spectrophotometer UV 300.

Results and Discussion

Number three in the Table, norflurazon [4-chloro-5-methylamino-2-(3-trifluoromethylphenyl)-pyrida-

Table I. I_{50} concentrations for interference of phenyl-substituted 4-chloro-5-methylamine-2-phenylpyridazin-3(2H) ones with greening of *Scenedesmus*.



No.	Substituents R at the phenyl ring	I_{50} [10^{-8} M]		σ_m	σ_p	π
		(experimental)	(calculated)			
1	3-SCF_3	0.4	1.1	0.40	0.50	1.44
2	3-OCF_2CHFCF_3	0.74	0.22	0.35[a]	0.28[a]	2.4[a]
3	3-CF_3	1	2.6	0.43	0.54	0.88
4	3,5-di-CF_3	30	–	2 × 0.43	2 × 0.54	2 × 0.88
5	4-CF_3	71	–	0.43	0.54	0.88
6	3-$OCHF_2$	8	7.6	0.31	0.18	0.85[a]
7	3-CN	8	18.2	0.56	0.66	− 0.57
8	3-Br	9.5	3.8	0.39	0.23	0.86
9	3-O-i-propyl	1	31	0.10	− 0.45	1.0
10	3-t-butyl	21	21	− 0.10	− 0.20	1.98
11	3-NO_2	40 [10][b]	–	0.71	0.78	− 0.28
12	3-H	180	562	0	0	0
13	3-OSO_2CH_3	360	141	0.39	0.36	− 0.88
14	3-OCH_3	1400	219	0.12	− 0.27	− 0.02

Hammett electronic parameters σ_m and σ_p and lipophilic parameters π were taken from [8].
[a] Values estimated in comparison with similar substituents.
[b] The corrected value is given in brackets, as explained in the text.

zin-3(2H)one] is the best bleaching pyridazinone known so far [2, 3, 6]. The activity of this compound was compared with the inhibition of chlorophyll accumulation (due to inhibition of carotene biosynthesis) by other 2-phenylpyridazinones in which an additional CF_3 group was inserted (No. 4) or in which the CF_3 group at the *meta* position of the phenyl moiety was either moved to *para* position (No. 5) or replaced by various other substituents with high positive Hammett electronic parameters.

Among these new phenylpyridazinones, five compounds, No. 1 with an SCF_3, No. 2 with OCF_2CHF_3 No. 6 with an $OCHF_2$ group, No. 7 carrying a CN, and No. 8 with a Br, all at *meta* position of the phenyl ring, exhibited strong inhibition of greening measured as decreased chlorophyll level. Their I_{50} values are either lower or in the same 10^{-8} M concentration range as of norflurazon.

When the CF_3 group was located in *para* instead of *meta* position (No. 5), a 70fold higher concentration was needed to obtain the same inhibition on chlorophyll accumulation as with norflurazon (No. 3). This indicates that the *meta* position is essential for activity-determining substituents. However, as seen with the OSO_2CH_3 derivative (No. 13), very low lipophilicity (expressed by π values) can counteract the bleaching activity expected by the high σ_m and σ_p values. Also in case of the cyano compound (No. 7), a low lipophilic parameter apparently is responsible for an 8 times higher I_{50} value than found with norflurazon, although, according to higher σ_m and σ_p values, bleaching activity better than with norflurazon was proposed [3]. On the other hand, the high π value of the SCF_3 group (No. 1) made this derivative the best bleaching phenylpyridazinone among the compounds of the Table, regardless of its smaller σ parameters.

Looking at the norflurazon-like derivative, No. 4, with an additions CF_3 moiety at position 5 of the phenyl ring (both CF_3 in *meta* position), it is conceivable that the bulkiness of the substituents can prevent the inhibitory activity of this phenylpyridazinone although an additive contribution should be expected.

Degradation of colored compounds during aeration in the light is another effect that has to be taken into account. This was observed with the nitro derivative (No. 11). The brownish color of this compound disappeared when it was added to the culture medium without cells at a concentration

of 0.3 mM (equivalent to an optical density of 0.21 at 390 nm). After 2 days of illumination and aeration, *i.e.* conditions normally used for the growth of algae, the concentration decreased to 68%. The experimental I_{50} value given in the Table should, therefore, be corrected down to at least 10×10^{-8} M.

On the basis of eleven *meta*-substituted 2-phenylpyridazinones from the Table (the 4-CF_4, 3,5-di-CF_3, and 3-NO_2 analogs were omitted), the following Hansch equation was calculated:

$$pI_{50} = 0.90(\pm 0.39)\,\pi + 3.57(\pm 1.91)\,\sigma_m + 5.25(\pm 0.73)$$

with a standard deviation $s = 0.52$, a significance $F = 19.2$, and a correlation coefficient of $r = 0.91$; $n = 11$. Other parameters available from the literature (*e.g.* Taft steric parameters) of the substituents did not show a significant contribution to this equation.

Inhibition of carotenogenesis by phenylpyridazinones substituted at *meta* position of the phenyl ring is influenced by lipophilic (π) and electronic (σ_m) properties of the substituents. However, the latter effect dominates over the first one. The calculated I_{50} values are included in the Table. Except for No. 14 (*m*-OCH_3), the experimental and calculated I_{50} values are quite close to each other (*e.g.* Nos. 6 and 10) or differ by a factor of up to three, which we regard as promising under the experimental conditions used.

It has to be considered that microalgae represent simple biological assay systems. Nevertheless, metabolic breakdown or chemical modifications of the analogs applied cannot be excluded. For correct activity determination compounds such as the *m*-OCH or *m*-NO_2 analogs should be subjected to a cell-free assay system using isolated carotene-synthesizing thylakoids which was developed recently [7].

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