QSAR of Nitro-diphenylethers as Inhibitors of Cyclic Photophosphorylation

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A number of 25 nitro-diphenylethers have been synthesized and assayed for their inhibitory activity in N-methylphenazonium-methosulfate mediated cyclic photophosphorylation. Their pI_{50}-values were in the range from 3.5 to 5.3. A QSAR-analysis revealed that the lipophilicity of the nitro-diphenylethers as expressed by $\Sigma\pi$ played a major role. In addition, the Taft steric parameter E_s in positions 6′, 4′ and 2′ (in decreasing order) contributed to the inhibitory activity. For maximal biological activity, positions 4′ and 6′ should be left unsubstituted whereas a bulky substituent is required for position 2′.

Introduction

Nitro-diphenylethers like nitrofen, oxyfluorfen or nitrofluorfen are used as efficient herbicides for weed control, especially in rice paddy cultures and soybeans. Their mode of action is assumed to be threefold. They inhibit photosynthetic electron transport, they interfere with photophosphorylation (energy transfer inhibition), and finally, they lead to peroxidative destruction of chloroplast components [1]. Inhibition of photosystem II electron transport by nitro-diphenylethers has been documented for quite a number of derivatives [1, 2]. In addition, a QSAR has been reported, where the lipophilicity parameter proved to be the most important one [2]. Furthermore, it was demonstrated that nitrofen competes with the triazine herbicide atrazine for a common binding site [3]. This locates the inhibition site of nitro-diphenylethers at the secondary quinone acceptor site of photosystem II (Q_B-site). The peroxidative activity of nitro-diphenylethers has also been extensively evaluated [1]. Contrary, there are much less and conflicting data available on the inhibition of photophosphorylation. Moreland *et al.* [4] find almost no inhibition of photophosphorylation by nitrofen and two other diphenylethers. An identical observation was made for nitrofluorfen [5]. In contrast, Lambert *et al.* report on inhibition of PMS-cyclic photophosphorylation by nitrofen [6] and several mono- and dichloro-nitro-diphenylethers [1]. By study of the exchange of tightly bound adenine nucleotides in the presence of nitrofen, it was concluded that nitrofen binds to the CF_1 subunit of chloroplast coupling factor. Energy transfer inhibition by nitrofen is due to this binding [3].

In view of the importance of nitro-diphenylether herbicides in agriculture and the limited data available for inhibition of cyclic photophosphorylation, we have synthesized 25 nitro-diphenylethers and tested them for their inhibitory activity in the latter system. All compounds were found to be moderate to good inhibitors, the best ones exhibiting pI_{50}-values up to 5.3. A QSAR could be established, where biological activity is correlated to the lipophilicity of the compound and in addition to steric parameters in certain positions.

Materials and Methods

Chemicals and synthesis

2-Nitro-4-trifluoromethyl-2′,4′-dichloro-* (No. 16) and 2-nitro-4,4′-dichloro-diphenylether (No. 14) were purchased from Aldrich (Alfred Baader Library of Rare Chemicals). Other nitro-diphenylethers were synthesized according to the references

Abbreviations: PMS, N-methyl-phenazonium-methosulfate; QSAR, quantitative structure-activity relationship.

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* Chemical nomenclature will not follow the normal rules. For reasons of the QSAR-analysis *nitro* substituents will preferentially be placed in positions 2–6.

cited in Table I. For dinitro-diphenylethers the following new synthesis protocol using phase transfer catalysis was applied:

11 mmol phenol was dissolved in 30 ml of 0.7 N NaOH. 1.86 g (10 mmol) 2,4-dinitro-fluorobenzene in 25 ml CH_2Cl_2 and 0.34 g (1 mmol) tetrabutylammonium-hydrogensulfate were added. The reaction mixture was stirred at room temperature for 24 h; the CH_2Cl_2-phase washed with H_2O, dried and the solvent evaporated in the vacuum. The solid residue was recrystallized from either cyclohexane or *n*-hexane/benzene. The identity of the compounds was checked by elementary analysis and/or mass spectrometry.

The following new dinitro-diphenylethers were synthesized (Nr. Tab. I/m.p. °C): 2,4-dinitro-4′-bromo-2′-isopropyl-5′-methyl (3/65), 2,4-dinitro-2′,4′-dibromo-6′-isopropyl-3′-methyl (4/106–108), 2,4-dinitro-2′,4′-diiodo-2′-isopropyl-5′-methyl (5/133), 2,4,4′-trinitro-2′-isopropyl-5′-methyl (8/85),

Table I. Parameters, experimental and calculated pI_{50}-values for inhibition of PMS-cyclic photophosphorylation by various nitro-diphenylethers.

No.	-diphenylether	$\Sigma\pi$	$E_s(2')$	$E_s(4')$	$E_s(6')$	pI_{50} found	pI_{50} calc.	Δ	Synthesis Ref.
1	2,4,4′-trinitro					<4[a]			[9]
2	2,4-dinitro-2′-cyclohexyl					<4[a]			[9]
3	2,4-dinitro-4′-bromo-2′-isopropyl-5′-methyl					<4[a]			[b]
4	2,4-dinitro-2′,4′-dibromo-6′-isopropyl-3′-methyl					<4[a]			[b]
5	2,4-dinitro-2′,4′-diiodo-2′-isopropyl-5′-methyl					<4[a]			[b]
6	2,4-dinitro-2′,4′,6′-triiodo	2.80	−1.08	−1.08	−1.08	3.55	3.63	0.08	[9]
7	2,4-dinitro	−0.56	0.32	0.32	0.32	4.42	4.43	0.01	[9]
8	2,4,4′-trinitro-2′-isopropyl-5′-methyl	1.81	−1.08	−2.20	0.32	4.43	4.43	±0	[b]
9	2,4-dinitro-3′,5′-diisopropyl	2.50	0.32	0.32	0.32	4.53	4.52	−0.01	[b]
10	4-nitro-4′-iodo	0.84	0.32	−1.08	0.32	4.76	4.89	0.13	[10]
11	2,2′,4-trinitro	−0.84	−2.20	0.32	0.32	4.79	4.78	−0.01	[9]
12	4-nitro-2′,4′-dichloro (nitrofen)	1.14	−0.65	−0.65	0.32	4.81	5.03	0.22	[11]
13	2,4-dinitro-4′-fluoro-2′,6′-dichloro	1.00	−0.65	−0.14	−0.65	4.84	4.89	0.05	[b]
14	2-nitro-4,4′-dichloro	1.14	0.32	−0.65	0.32	4.85	4.99	0.14	[b]
15	2,4-dinitro-2′-chloro-4′-bromo	1.01	−0.65	−0.84	0.32	4.86	5.00	0.14	[9]
16	2-nitro-4-trifluoromethyl-2′,4′-dichloro	2.02	−0.65	−0.65	0.32	4.88	4.80	−0.08	[b]
17	2,4-dinitro-4′-fluoro-2′,6′-dibromo	1.30	−0.84	−0.14	−0.84	4.91	4.74	−0.17	[b]
18	2,4-dinitro-2′-chloro	0.71	−0.65	0.32	0.32	4.95	5.05	0.10	[9]
19	4-nitro-2′-chloro	0.43	−0.65	0.32	0.32	4.97	4.99	0.02	[12]
20	2,4-dinitro-4′-fluoro-3′-chloro	0.29	0.32	−0.14	0.32	4.97	4.92	−0.05	[b]
21	2,4-dinitro-4′-trifluoro-methyl-2′-azido	0.78	[c]			5.02			[b]
22	2,4-dinitro-2′-isopropyl	0.97	−1.08	0.32	0.32	5.08	5.17	0.09	[9]
23	2,4-dinitro-4-iodo	0.56	0.32	−1.08	0.32	5.15	4.85	−0.30	[9]
24	2,4-dinitro-2′-isopropyl-5′-methyl	1.53	−1.08	0.32	0.32	5.19	5.11	−0.08	[9]
25	2,4-dinitro-2′,4′-dichloro	1.42	−0.65	−0.65	0.32	5.29	5.00	−0.29	[9]

The parameters were calculated from the values given in [13].
[a] Exact determination of pI_{50}-value was not possible due to low solubility.
[b] See Materials and Methods.
[c] E_s-value for azido group is not known.

2,4-dinitro-3′,5′-diisopropyl (9/97), 2,4-dinitro-4′-fluoro-2′,6′-dichloro (13/137), 2,4-dinitro-4′-fluoro-2′,6′-dibromo (17/145), 2,4-dinitro-4′-fluoro-3′-chloro (20/96) and 2,4-dinitro-4′-trifluoromethyl-2′-azido (21/86).

Biochemical methods

Chloroplasts from spinach were prepared according to [7]. PMS-cyclic photophosphorylation was measured as described in [8].

Results and Discussion

Table I lists the pI_{50}-values for the nitro-diphenylethers tested, which lie in the range from 3.55 to 5.29. For compounds 1–5 the exact pI_{50}-values could not be determined because they precipitated upon addition to the assay system. An azido-derivative (Nr. 21) as a possible candidate for a radioactive photoaffinity label was included in the series. It should be noted that the nitro-diphenylethers from Table I are also weak inhibitors of photosystem II electron transport (data not shown) which has also been demonstrated for other nitro-diphenylethers [1, 2, 6]. Compounds 4 and 5 in addition are strong inhibitors of electron flow through the cytochrome b_6/f complex (pI_{50}-values of 6.75 and 6.85, respectively, in the system durohydroquinone/methylviologen). This is not surprising because these two compounds are closely related to the dinitrophenylethers of bromo- or iodonitrothymol, which have been reported to inhibit electron flow through the cytochrome b_6/f region [14].

For a QSAR analysis of the inhibition data a parameter set consisting of the following parameters was constructed: the sum of the sigma-Hammett values ($\Sigma\sigma$) for positions 2–6, same for positions 2′–6′, the sum of the lipophilicity constants ($\Sigma\pi$), and Taft's steric parameters (E_s, based on methyl) for positions 2, 4, 2′, 3′, 4′ and 6′. In addition, the square terms of the parameters mentioned were included. In the statistical analysis (using a statistics package on a personal computer) first a correlation matrix between all parameters and the pI_{50}-values was established. The highest correlation (−0.677) was found for $(\Sigma\pi)^2$. In a stepwise multiple regression analysis first the equation between $(\Sigma\pi)^2$ and pI_{50}-values was calculated. The program will then add automatically parameters to the regression by selecting for the highest F-prime value. The following parameters (with decreasing F-value) have been chosen: $\Sigma\pi$, $(E_s(6'))^2$, $(E_s(4'))^2$ and $(E_s(2'))^2$, yielding this equation (Eqn. (1)):

$$pI_{50} = 4.842 + 0.497\,\Sigma\pi - 0.241\,(\Sigma\pi)^2 + 0.124\,(E_s(2'))^2 - 0.126\,(E_s(4'))^2 - 0.607\,(E_s(6'))^2 \quad (1)$$

$n = 19$, $r = 0.929$; $s = 0.145$;
$F = 16.45$ ($F_{13,5} = 4.86$, $\alpha = 0.01$).

The pI_{50}-values calculated according to this equation and the differences between values found and calculated are listed in Table I. A plot of found *versus* calculated pI_{50}-values is shown in Fig. 1. The correlation matrix together with the t-tests for the parameters is given in Table II. Except for a high

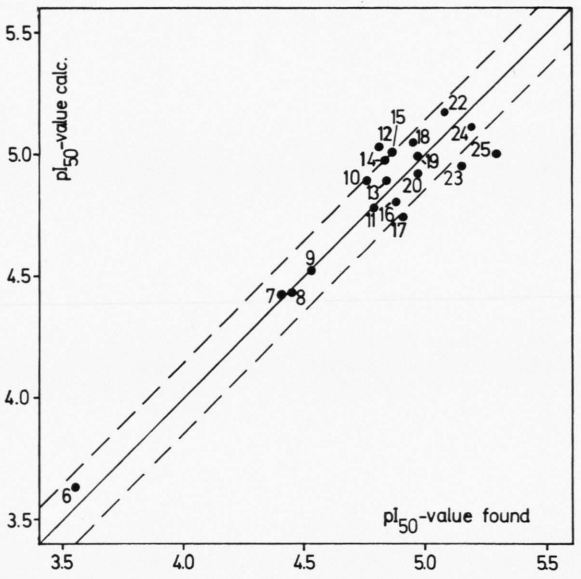

Fig. 1. Plot of pI_{50}-value found *versus* pI_{50}-value calculated according to Eqn. (1). The dashed lines indicate the standard deviation.

Table II. Correlation matrix and t-test for the parameters used in Eqn. (1).

	$\Sigma\pi$	$(\Sigma\pi)^2$	$(E_s(2'))^2$	$(E_s(4'))^2$	$(E_s(6'))^2$
$\Sigma\pi$	1.000	0.841	−0.324	0.288	0.439
$(\Sigma\pi)^2$		1.000	0.016	0.241	0.560
$(E_s(2'))^2$			1.000	0.030	0.068
$(E_s(4'))^2$				1.000	0.010
$(E_s(6'))^2$					1.000
t-test	4.732	−5.528	2.622	−3.305	−3.546

correlation between $\Sigma\pi$ and $(\Sigma\pi)^2$, which is reasonable, the parameters are not intercorrelated.

The biological activity of the nitro-diphenylethers is first described by their lipophilicity and the square term of it, indicating that (i) the lipophilicity of the compound plays a major role and (ii) that the maximum of lipophilicity has already been reached within the starting compound set. Hence, for other compounds of this series no better biological activity *in vitro* can be expected. However, in addition to the lipophilicity an influence of the steric parameter, but only in special positions (6′, 4′, 2′), on biological activity is relevant. For Eqn. (1) the square terms of the E_s-parameters have been selected by the program, and they have negative signs for $E_s(6')$ and $E_s(4')$, but a positive sign for $E_s(2')$. This means that for maximal biological activity the 6′- and 4′-positions should be left unsubstituted or substituted only by a small substituent, whereas a bulky substituent is required for position 2′.

As already stressed, the nitro-diphenylethers are used as powerful herbicides. The inhibition of photophosphorylation by them is only moderate with maximal pI_{50}-values of ≈ 5.3. It seems rather doubtful whether inhibition of photophosphorylation contributes to their *in vivo* activity.

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