Quantitative Structure Activity Relationship of Diphenylamines as Inhibitors of Photosynthetic Electron Transport and Photophosphorylation

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Diphenylamines, Inhibitors, Photosynthetic Electron Transport

A number of 16 substituted diphenylamines have been tested for their inhibitory activity on photosynthetic NADP reduction and photophosphorylation. The most active compounds exhibited pI_{50} values of 6.0 in photosynthetic electron transport and 6.8 in cyclic photophosphorylation, respectively. The inhibition site in electron flow of the diphenylamines is located between the two photosystems. Necessary for high activity is the substitution of the phenyl moieties by several strongly electron withdrawing substituents. A quantitative structure activity relationship according to a parabolic Hansch approach could be accomplished by using the Hammett electronic parameter as the only variable.

Introduction

Substituted diphenylethers are widely used as efficient rice herbicides in Japan (for a recent review see ref. [1]). In a study on their mechanism of action, Moreland *et al.* [2] have tested their effects on biochemical reactions of isolated chloroplasts and mitochondria. They reported inhibition of photosynthetic NADP reduction (pI_{50} values 4.5 – 5.2), cyclic photophosphorylation (pI_{50} values less than 3.5) and of respiration in mitochondria (pI_{50} values 4.1 – 4.8 for different substrates) [2].

The dinitrophenylethers of bromo- and iodo-nitrothymol have been very recently introduced by Trebst *et al.* [3] as powerful inhibitors of photosynthetic electron transport. Their pI_{50} values of 6.5 and 7.0, respectively, exceed the pI_{50} values of the so long known diphenylethers by almost two orders of magnitude. Their site of inhibition is identical with that of DBMIB [3].

In search for new potential herbicides we have investigated structural analogues of diphenylethers like diphenylamines. They indeed proved to be inhibitors of photosynthetic electron transport with pI_{50} values of up to 6.0. In addition, they were also inhibitors of cyclic photophosphorylation.

Materials and Methods

The compounds from Table I were obtained from the following sources: diphenylamine (**2**) from Merck AG, Darmstadt; 3-methyl- (**4**), 3-chloro- (**5**), 3-trifluoromethyl- (**6**), 2-nitro- (**9**) and 2,4,6,2′,4′,6′-hexanitro-diphenylamine from Aldrich Europe, and 3-trifluoromethyl-2′,4′,6′-trinitro- (**10**), 3-fluoro-2′4′, 6′-trinitro- (**11**), 2,4,6-trichloro-2′,4′,6′-trinitro- (**12**), 4-iodo-2′,4′,6′-trinitro- (**13**), 2,4-dibromo-2′,4′,6′-trinitro- (**14**), and 2-chloro-5-trifluoromethyl-2′,4′,6′-trinitro-diphenylamine (**16**) from Alfred Bader Chemicals, Library of Rare Chemicals, Aldrich. 3-Hydroxy-2-methyl-diphenylamine (**1**) was purchased from Sandoz AG, Basle, Switzerland, and converted to 2-methyl-3-methoxy-diphenylamine (**3**) by treatment with diazomethane. 2,4-Dinitro- (**8**) and 2,4-dinitro-2′,4′,6′-trimethyl-diphenylamine (**7**) were synthesized by reaction of 2,4-dinitrofluorobenzene with the corresponding amines analogous a procedure by Reinheimer *et al.* [4]. 2,4,6,2′,4′,6′-Hexanitrodiphenylamine (dipycrylamine) was used as its potassium salt [5].

The inhibitory activity of the diphenylamines was tested in photosynthetic NADP reduction with water as the electron donor under uncoupled conditions and pI_{50} values determined as described recently [6].

Cyclic photophosphorylation was measured under aerobic conditions. The reaction mixture contained in a final volume of 1 ml in μmol: Tricine, pH 8.0, 50; NaCl 50; $MgCl_2$ 5; ADP 3; P_i containing about 10^6 cpm ^{32}P 2; Na-ascorbate 2; PMS 0.1; and

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Abbreviations: DAD, diaminodurene; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; DBMIB, 2,5-dibromo-3-methyl-6-isopropyl-1,4-benzoquinone; PMS, N-methylphenazonium methosulfate; QSAR, quantitative structure activity relationship.

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Table I. Experimental pI$_{50}$-values for electron transport and photophosphorylation, Hammett electronic parameters $\Sigma\,\sigma$, calculated pI$_{50}$-values for electron transport, and difference between experimental and calculated pI$_{50}$-values for various diphenylamines

Nr.	-diphenylamine	pI$_{50}$ [a] e-transport	pI$_{50}$ [a] ATP	$\Sigma\,\sigma$ [b]	pI$_{50}$ calc.	ΔpI$_{50}$
1	3-hydroxy-2-methyl-	3.88	4.18	−0.05	4.08	−0.20
2	—	3.99	4.43	0	4.12	−0.13
3	2-methyl-3-methoxy-	4.07	3.89	−0.05	4.08	−0.01
4	3-methyl-	4.13	3.50	−0.07	4.07	0.06
5	3-chloro-	4.49	4.88	0.37	4.39	0.10
6	3-trifluoromethyl-	4.68	4.87	0.43	4.43	0.25
7	2,4-dinitro-2′,4′,6′-trimethyl-	4.69	4.65	1.05	4.83	−0.14
8	2,4-dinitro-	4.91	4.22	1.56	5.11	−0.20
9	2-nitro-	4.92	4.37	0.78	4.67	0.25
10	3-trifluoromethyl-2′,4′,6′-trinitro-	5.32	6.74	2.77	5.61	−0.29
11	3-fluoro-2′,4′,6′-trinitro-	5.43	5.00	2.68	5.58	−0.15
12	2,4,6-trichloro-2′,4′,6′-trinitro-	5.57	5.15	3.03	5.68	−0.11
13	4-iodo-2′,4′,6′-trinitro-	5.62	6.68	2.52	5.52	0.10
14	2,4-dibromo-2′,4′,6′-trinitro-	5.80	6.60	2.80	5.62	0.18
15	2,4,6,2′,4′,6′-hexanitro-	5.90	6.20	4.68	5.90	0
16	2-chloro-5-trifluoromethyl-2′,4′,6′-trinitro-	5.97	6.60	3.00	5.67	0.30

[a] At a concentration of chloroplasts with 14 μg chlorophyll.

[b] σ values were taken from Hansch *et al.* [9].

chloroplasts [7] corresponding to 14 μg of chlorophyll. The samples were illuminated in a water bath at room temperature for 3 min with white light at an intensity of 10^6 erg sec^{-1} cm^{-2}. Esterified ^{32}P$_i$ was assayed according to McCarty and Racker [8].

[^{14}C]metribuzin was a generous gift from Dr. W. Draber, Bayer AG, Wuppertal. For displacement experiments, chloroplasts corresponding to 100 μg chlorophyll in 2 ml 20 mM tricine buffer, pH 8.0, were incubated for 5 min with 10^{-7} M [^{14}C]metribuzin. Then the diphenylamine was added, again incubated for 5 min, and the chloroplasts removed by filtration through a SM 11 305 membrane filter, pore size 0.65 μm. The chloroplasts were washed once with 2 ml buffer, the membrane filter with the chloroplasts dissolved in 10 ml aquasol (Lumac GmbH), and radioactivity counted in a Packard Tricarb Liquid Scintillation Spectrometer, model 3385.

Results and Discussion

For an assay of their biochemical activity 16 diphenylamines have been available. They have been tested for their inhibitory activity on photosynthetic NADP reduction in chloroplasts. Water has been used as the electron donor and the system has been uncoupled. In Table I, column 1, pI$_{50}$ values for inhibition of NADP reduction are listed in the order of increasing magnitude. In addition, the influence of diphenylamines on PMS-mediated cyclic photophosphorylation has also been determined. As can be seen from Table I, column 2, diphenylamines are effective inhibitors of photophosphorylation too. Both activities, *i. e.* inhibition of electron transport or phosphorylation, are not closely related to each other. In some cases the pI$_{50}$ value of inhibition in electron transport exceeds that of inhibition in photophosphorylation (for example, 2,4-dinitro-diphenylamine (**8**) 4.91 and 4.22, respectively), in other cases the opposite is true (for example, 3-trifluoromethyl-2′,4′,6′-trinitrodiphenylamine (**10**) 5.32 and 6.74, respectively). Inhibitors of this type have been termed inhibitory uncouplers by Moreland and Hilton [10].

A close examination of the pI$_{50}$ values in Table I reveals that inhibitory activity increases with the number and the electronegativity of the substituents. The best inhibitors are compounds with 5 or 6 electronegative substituents attached to the phenyl rings, amongst which halogeno-, trifluoromethyl- and nitro-groups proved to be the most effective.

In Table I the sum of the Hammett electronic parameters $\Sigma\,\sigma$ of each compound are listed as measure of the sum of the electronegativity of the substituents. $\Sigma\,\sigma$ has been calculated by using the appropriate σ parameters for the *p*- and *m*-positions. As can be seen from Fig. 1, there exists an almost

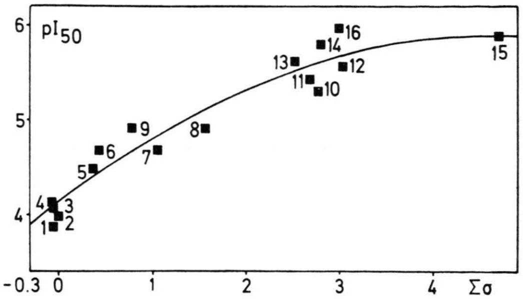

Fig. 1. Relationship between pI_{50} values and sum of Hammett electronic parameters $\Sigma\sigma$ for diphenylamines from Table I.

linear dependency of pI_{50} values in electron transport from $\Sigma\sigma$. Multiple linear regression gave an optimal fit in QSAR by a slightly curved parabola of the equation:

$$pI_{50} = 4.121 + 0.762\,\Sigma\sigma - 0.082\,(\Sigma\sigma)^2$$
$$n = 16, \quad r = 0.967, \quad s = 0.184, \quad F = 99.0\,.$$

In Table I are also given the pI_{50} values calculated according to the above equation and the differences between the calculated and experimental pI_{50} values. A similar QSAR for pI_{50} values in photophosphorylation and $\Sigma\sigma$ has also been attempted. The correlation coefficient, however, was worse then above and some compounds showed a deviation of more than 1 between calculated and experimental values.

QSAR studies on inhibitors of photosynthetic electron transport have been mainly performed on inhibitors of the DCMU type, which are commercially used as herbicides. In these cases, mostly the apparent dissociation constant pk_a, partition coefficient P, Hammett σ value and Taft's steric constant E_s have been used as parameters [11 – 16]. Normally, a QSAR with high correlation coefficient could be obtained by employing only one parameter. The same was true for DBMIB type inhibitors where only the lipophilicity parameter π plays an important role [6].

In this respect diphenylamines are exceptional because a QSAR with high correlation coefficient could be obtained by use of only the Hammett σ parameter. This is not the case for all other electron transport inhibitors. From the dependency of inhibitory activity of diphenylamines from the electronegativity of the substituents at the aromatic moiety it has to be concluded that the $-\overline{N}-H$ group plays the central role for aspects of the mechanism of action. With increasing $\Sigma\sigma$ value of the substituents the lone electron pair at the nitrogen atom will be more delocalized over the entire aromatic system and in consequence the hydrogen atom will get more acidic. This is reflected in the pk_a values. As has been determined for some diphenylamines, pk_a value will drop with increasing $\Sigma\sigma$. In aqueous solution, dipicrylamine (**15**) ($pk_a = 2.3$) preferentially exists as its anion [5].

For some diphenylamines the dependency of pI_{50} values from chlorophyll concentration has been determined. As will be reported in detail elsewhere, pI_{50} values of 2,4,6,2',4',6'-hexanitro-(**15**) and 2-chloro-5-trifluoromethyl-2',4',6'-trinitro-diphenylamine (**16**) are strongly chlorophyll-dependent, whereas pI_{50} value of 2,4,6-trichloro-2',4',6'-trinitro-diphenylamine (**12**) is not. According to Tischer and Strotmann [17] I_{50} values extrapolated for zero chlorophyll concentration are identical with the binding constants. Thus, a binding constant of 0.107 μM

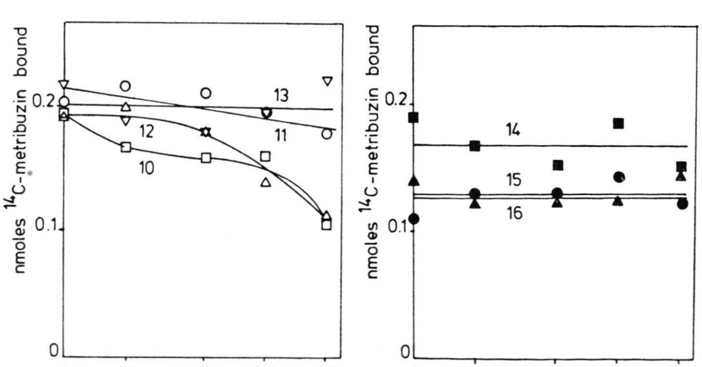

Fig. 2. Competitive displacement of [^{14}C]metribuzin from chloroplast membranes by various diphenylamines. For conditions see Materials and Methods.

has been determined for 2,4,6,2′,4′,6′-hexanitro-diphenylamine (**15**) and of 0.172 μM for 2-chloro-5-trifluoromethyl-2′,4′,6′-trinitro-diphenylamine (**16**).

The inhibition site of diphenylamines is tentatively localized between the two photosystems. As will be reported elsewhere, this is concluded from the fact that photosystem I dependent reactions are not inhibited or only to a small extent. Furthermore, the diphenylamines do not share an identical inhibition site with DBMIB. The inhibition site is also different from that of DCMU. This could be established from the fact that [^{14}C]metribuzin was not displaced from chloroplast membranes by diphenylamines.

This experiment for diphenylamines **10** to **16** is shown in Fig. 2. Only compounds **10** and **12** at very high concentration displace [^{14}C]metribuzin from the chloroplast membrane to a small extent, whereas all others do not. According to Tischer and Strotmann [17] this result indicates for diphenylamines a different binding and inhibition site then metribuzin, which itself has been proven to be an inhibitor of the DCMU type.

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[1] S. Matsunaka in Herbicides, Chemistry, Degradation, and Mode of Action (P. C. Kearney, and D. D. Kaufman, eds.), **Vol. 2,** p. 709, Marcel Dekker, Inc. New York and Basle 1976.

[2] D. E. Moreland, W. J. Blackmon, H. G. Todd, and F. S. Farmer, Weed Sci. **18,** 636 (1970).

[3] A. Trebst, H. Wietoska, W. Draber, and H. J. Knops, Z. Naturforsch. **33c,** 919 (1978).

[4] J. D. Reinheimer, J. P. Douglass, H. Leister, and M. B. Voelkel, J. Org. Chem. **22,** 1743 (1957).

[5] S. Motomizu, K. Toei, and T. Iwachido, Bull. Chem. Soc. Japan **42,** 1006 (1969).

[6] W. Oettmeier, S. Reimer, and K. Link, Z. Naturforsch. **33c,** 695 (1978).

[7] N. Nelson, Z. Drechsler, and J. Neumann, Biol. Chem. **245,** 143 (1970).

[8] R. E. McCarty and E. Racker, J. Biol. Chem. **242,** 3435 (1967).

[9] C. Hansch, A. Leo, S. H. Unger, K. H. Kim, D. Nikaitani, and E. J. Lien, J. Med. Chem. **16,** 1207 (1973).

[10] D. E. Moreland, and J. L. Hilton, Herbicides, Physiology, Biochemistry, Ecology (L. J. Audus, ed.), **Vol. 1,** p. 493, Academic Press, London, New York, San Francisco 1976.

[11] C. Hansch, Progress in Photosynthesis Research (H. Metzner, ed.), **Vol. 3,** p. 1685, Tübingen 1969.

[12] A. Trebst and W. Draber, Advances in Pesticide Science (H. Geissbühler, ed.), Part **2,** p. 223, Pergamon Press, Oxford and New York 1979.

[13] N. D. Camper and D. E. Moreland, Biophys. Acta **94,** 383 (1965).

[14] K. H. Büchel, Pestic. Sci. **3,** 89 (1972).

[15] W. Draber, K. H. Büchel, H. Timmler, and A. Trebst, Mechanism of Pesticide Action, ACS Symposium Series, Number **2,** 100 (1974).

[16] W. Draber, K. H. Büchel, and K. Dickoré in Proceedings of the Second International IUPAC Congress of Pesticide Chemistry (A. S. Tahori, ed.), **Vol. 5,** p. 153, Gordon and Breach Science Publishers, New York, London and Paris 1972.

[17] W. Tischer and H. Strotmann, Biochim. Biophys. Acta **460,** 113 (1977).