Quantitative Structure-Activity Relationship of Substituted Benzoquinones as Inhibitors of Photosynthetic Electron Transport *

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1.4-benzoquinones have been tested for their inhibitory activity on photosynthetic $NADP^+$ reduction by chloroplasts. Benzoquinones with one or two branched alkyl side chains are inhibitors of electron flow. Inhibitory activity can be highly increased if one — and even more if two — halogen substitutents are introduced into the quinone moiety. Iodine substitution yields a better inhibitor than bromine than chlorine. A quantitative structure activity relationship according to a bilinear model, as developed by Kubinyi, with the lipophilicity as the only parameter could be established.

2,5-Dibromo-3-methyl-6-isopropyl-1,4-benzoquinone (DBMIB) has been introduced by Trebst *et al.* [1] as a powerful inhibitor of photosynthetic electron transport. It is acting as a plastoquinone antagonist and inhibits electron flow between plastoquinone and plastocyanin. This allows studies of electron transport phenomena and photophosphorylation, which are either connected to photosystem I or photosystem II [2, 3].

Besides DBMIB, several other quinones, mostly benzoquinones substituted by long alkyl side chains, and naphtoquinones are known as inhibitors of photosynthetic electron transport [2, 4–7] and photophosphorylation in bacterical chromatophores as well [8, 9]. In photosynthetic electron transport they too inhibit between the two light reactions, but their site of inhibition is not identical with and less specific than that of DBMIB, but closer to the reducing site of photosystem II [6, 7]. Furthermore, their inhibitory activity is about a hundredfold less as compared to DBMIB [2, 4–7].

So far, the structure activity relationship of halogenated benzoquinones has not been throughly investigated. We wish to report here on our results on the inhibitory potency of substituted quinones. In order to get an inhibitor of the DBMIB type, a halogen substitution at the quinone moiety is not absolutely necessary. Highly lipophilic alkylsubstituted 1,4-benzoquinones are already good inhibitors. Additional introduction of halogen substitution in general increases activity; two halogens being more effective than one. A tendency towards better inhibitory activity follows the sequence:

chlorine < bromine < iodine.

Materials and Methods

1. Source and synthesis of quinones and hydroquinones

2,6-Di-*tert*-butyl-1,4-benzoquinone (**1 g**), tetrafluoro-1,4-benzoquinone (**4 a**), trimethyl-hydroquinone, *tert*-butyl-hydroquinone, and 2,5-*tert*-butyl-hydroquinone were obtained from Aldrich. Merck-Schuchardt was the source for tetramethyl-1,4-benzoquinone (**1 d**) and tetrabromo-1,4-benzoquinone (bromanil) (**4 c**), and Fluka, Switzerland, for tetrachloro-1,4-benzoquinone (chloranil) (**4 b**). 2,6-Dibromo-3-methyl-5-isopropyl- (**2 i**), 2-bromo-3,5-diisopropyl- (**2 j**), and 2,6-dibromo-3,5-diisopropyl-1,4-benzoquinone (**2 k**) were a generous gift by Dr. W. Draber and will be described elsewhere.

Trimethyl- (**1 c**), *tert*-butyl- (**1 e**), and 2,5-di-*tert*-butyl-1,4-benzoquinone (**1 f**) were prepared from the corresponding hydroquinones by oxidation with chromic acid in acetic acid. 2-Methyl-5-isopropyl-hydroquinone and tetramethyl-hydroquinone were obtained by reduction of 2-methyl-5-isopropyl-1,4-benzoquinone (thymoqinone) (**1 a**) [11] or tetramethyl-1,4-benzoquinone (**1 d**), respectively, with sodium borohydride in ethanol.

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Abbreviations: DBMIB, 2,5-dibromo-3-methyl-6-isopropyl-1,4-benzoquinone; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; QSAR, quantitative structure activity relationship; TMPD, N,N,N′,N′-tetramethyl-*p*-phenylenediamine.

Reduction of the tetrahalogen-1,4-benzoquinones **4 a – 4 d** to the corresponding hydroquinones was performed by catalytic hydrogenation in acetone with palladium/charcoal or platinum as catalyst.

Synthesis of the quinones listed in Table I, 1 – 4 and the compounds from Table II as far as they are known in literature, was carried out according to the references given therein. Identity and purity of the compounds synthesized were checked by melting point, elementary analysis, NMR-spectra, and gaschromatography (Varian Aerograph, series 1400, column 5 ft, 1/4′, 5% silicon SE-30 on Chromosorb WAW DMCS 60/80, connected to an integrator, model 477).

For the following quinones, new synthetic procedures have been developed or they have been synthesized for the first time.

2,5-Dibromo-3,6-diphenyl-1,4-benzoquinone (**2 a**). 2.60 g (10 mmol) 2,5-diphenyl-1,4-benzoquinone (Eastman) are heated with 70 ml 40% HBr/acetic acid at 90 °C for 4 h. The mixture is then poured into water and the residue filtered off. It is oxidized without further purification by dissolving it in 50 ml acetic acid and adding 5 g of CrO_3 in 20 ml acetic acid and 10 ml H_2O. After pouring the reaction mixture into water, the precipitate is filtered and recrystallized from ethanol; yield 0.70 g (17%), m.p. 226 – 28 °C.

$C_{18}H_{10}Br_2O_2$ (418.1)

Calc. % C 51.71 H 2.41 Br 38.23
Found % C 52.51 H 2.54 Br 37.85

2,3,5-Tribromo-6-n-hexyl-1,4-benzoquinone (**2 d**). To 1.65 g (8.6 mmol) 2-*n*-hexyl-1,4-benzoquinone [12] in 8.3 ml acetic acid are added 1.5 ml bromine and 3.3 g anhydrous sodium acetate. The reaction mixture is kept at 90 °C for 1 h, poured into water, and the precipitate is filtered and dried; yield 2.50 g (68%). Recrystallized from ethanol; m.p. 61 – 63 °C.

$C_{12}H_{13}Br_3O_2$ (429.0; found masspectrometrically: 429)

Calc. % C 33.60 H 3.05 Br 55.89
Found % C 33.98 H 3.10 Br 55.30

2-Bromo-5-tert-butyl-1,4-benzoquinone (**3 a**). To 8.20 g (50 mmol) of 2-*tert*-butyl-1,4-benzoquinone (**1 e**) are added 60 ml of 40% HBr/acetic acid under vigorous stirring. The mixture is allowed to stand at room temperature for 1 h. Excess HBr is removed by bubbling air through the mixture. The 2-bromo-5-*tert*-butyl-benzohydroquinone formed is oxidized without further purification by addition of a solution of 5 g of CrO_3 in 20 ml of acetic acid and 10 ml of H_2O. The reaction mixture is poured into 1.3 l of H_2O and the precipitate collected by filtration; yield 5.60 g (46%). Recrystallized from ethanol, m.p. 106 °C (m.p. 110 – 112 °C [13]).

2.3-Dichloro-5-tert-butyl-1,4-benzoquinone (**3 b**) was prepared according to J. A. Van Allan *et al.* [14] as described for the synthesis of 2,3-dichloro-5,6-dimethyl-1,4-benzoquinone. Yield 89%; recrystallized from ethanol, m.p. 88 °C (89 – 89.5 °C [15]).

2-Iodo-3-hydroxy-5-tert-butyl-1,4-benzoquinone (**3 e**). To 2.08 g (5 mmol) of 2,3-diiodo-5-*tert*-butyl-1,4-benzoquinone (**3 d**) [16] in 750 ml methanol and 400 ml H_2O 37 ml 1 N NaOH are added. The solution is stirred at room temperature for 1 h, concentrated in the vacuum and acidified by addition of 6 N HCl. The precipitate is filtered off and dried; yield 1.02 g (65%). Recrystallized from petrol ether, boiling range 60 – 80 °C; m.p. 176 °C (dec.).

$C_{10}H_{11}JO_3$ (306.1; found masspectrometrically 306)

Calc. % C 39.24 H 3.62 J 41.46
Found % C 40.18 H 3.65 J 40.40

The structure of 2-iodo-3-hydroxy-5-*tert*-butyl-1,4--benzoquinone is not yet conclusively established. The product isolated from the reaction above may also be the isomeric 3-iodo-2-hydroxy-5-*tert*-butyl-1,4-benzoquinone or a mixture of both isomers.

2,5-Dibromo-3,6-di(isopropyloxy)-1,4-benzoquinone (**4 f**) was prepared as described by Wallenfels and Draber [17] for the corresponding chloro compound. Yield 89%; recrystallized from dimethylformamide; m.p. 161 – 64 °C.

$C_{12}H_{16}Br_2N_2O_2$ (380.1)

Calc. % C 37.92 H 4.24 Br 42.05 N 7.37
Found % C 38.09 H 4.23 Br 42.09 N 7.39

2,5-Dibromo-3,6-di(cyclohexylamino)-1,4-benzoquinone (**4 h**). Like **4 g**, yield 81%. Recrystallized from dimethylformamide; m.p. 155 °C dec.).

$C_{18}H_{24}Br_2N_2O_2$ (460.2)

Calc. % C 46.97 H 5.26 Br 34.73 N 6.09
Found % C 47.25 H 5.17 Br 34.69 N 5.96

2,5-Dibromo-3-methyl-6-isopropyl-1,4-benzoquinone-dimethyl-ether (**5 b**). 3.50 g (10.8 mmol) 2,5-dibromo-3-methyl-6-isopropyl-1,4-benzohydroquinone [18] in 20 ml ether are treated with an excess of diazomethane in ether. After standing for 1 h, the ether is evaporated in the vacuum. For removal of quinone, which might have been formed by oxidation of the hydroquinone, the residue is steam destilled and then taken up again in ether. After extraction with 2 N NaOH and drying over $MgSO_4$, the ether is evaporated and the remaining oil destilled in the vacuum (b.p.$_{0.4}$ 90 °C). A small sample is chromatographed on silicagel (petrol ether/acetone 1:1) and the major zone eluted.Masspectrometrical analysis showed it to be the dimethylether (M^+ 352).

2. Determination of pI_{50}-values

pI_{50}-values ($-\log_{10}$ of molar concentration giving 50% inhibition) were determined by the inhibition of photosynthetic NADP-reduction.

Spinach chloroplasts were prepared according to Nelson *et al.* [19]. They were frozen and stored in liquid nitrogen in the presence of 10% glycerol.

Photosynthetic $NADP^+$ reduction was measured at 340 nm in a Zeiss PMQ III spectrophotometer modified for illumination with red light (645 nm) at an intensity of 2.5×10^5 erg cm^{-2} sec^{-1}. The reaction mixture contained in a volume of 2 ml in μmoles: tricine-NaOH, pH 8.0, 40; $MgCl_2$, 10; NH_4Cl, 10; NADP, 3; ferredoxin from spinach, 0.01; and chloroplasts with 14 μg chlorophyll. Quinones were added in methanolic solution at a concentration that the concentration of methanol in the reaction mixture did not exceed 0.5%. Rates of $NADP^+$ reduction were measured at different concentrations of every quinone and extrapolated graphically to the pI_{50}-value.

Results

The quinones, which are either commercially available or have been synthesized, were tested for their inhibitory activity on photosynthetic $NADP^+$-reduction. Their inhibitory potency is compared for elucidation of the basic structural element for inhibitory activity.

1. Influence of the alkyl substitution on inhibitory activity

Normally, simple 1,4-benzoquinones, *i. e.* quinones substituted by small alkyl or alkoxy groups are not inhibitors but artificial electron acceptors of either photosystem I [31, 32] or photosystem II [33] as is known since 30 years.

This acceptor property is true for the quinones listed in Table I. 1, but in addition they are also inhibitors. This can be seen from the data in Fig. 1 A. After addition of 5×10^{-5} M 2-*tert*-butyl-1,4-benzoquinone (**1 e**), photosynthetic $NADP^+$ reduction not only stops, but the NADPH formed upon previous illumination is slowly reoxidized by the quinone, which gets reduced to the hydroquinone. Upon further illumination the quinone as artificial acceptors gets preferentially reduced by the chloroplasts at an inhibited rate, because it acts as its own inhibitor. When the quinone is reduced completely, $NADP^+$ reduction starts again but at a rate which is lower than the control, *i. e.* inhibited by 77% as compared to the original rate. Because the acceptor property of the quinone may obscure inhibitory activity, the quinones from Table I. 1 which may act as acceptors have been tested as hydroquinones. In this case no side reactions take place and the inhibited rate of photosynthetic $NADP^+$ reduction is observed immediately after addition of the hydroquinone (Fig. 1 B).

In the following tables, besides pI_{50}-values also hydrophobic parameters, calculated pI_{50}-values and differences between experimental and calculated

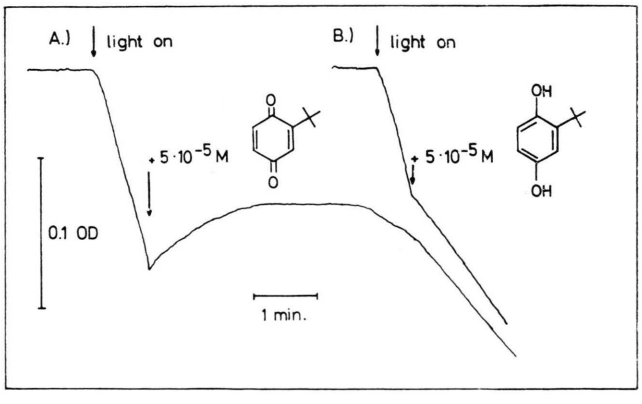
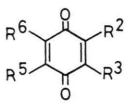


Fig. 1. Inhibition of photosynthetic $NADP^+$ reduction by 2-*tert*-butyl 1,4-benzoquinone and 2-*tert*-butyl-1,4-hydroquinone. Conditions as in Materials and Methods. Downward slope means increase in optical density.

Table I. Experimental pI_{50}-values, hydrophobic parameters ($\Sigma\pi_{2,3,5,6}$), calculated pI_{50}-values and difference between experimental and calculated pI_{50}-values (ΔpI_{50}) for various 1,4-benzoquinones.



No.	R^2	R^3	R^5	R^6	pI_{50}	TMPD bypass	$\Sigma\pi_{2,3,5,6}$	pI_{50} calc.	ΔpI_{50}	Synthesis Ref.
1. Alkylsubstituted 1,4-benzoquinones										
1 a	CH_3	H	$(CH_3)_2CH$	H	<4 [b]		2.09	4.87		a
1 b	$CH_3(CH_2)_5$	H	H	H	<4		3.00	6.04		[12]
1 c	CH_3	CH_3	CH_3	H	4.57 [b]	−	1.67	4.31	0.26	a
1 d	CH_3	CH_3	CH_3	CH_3	5.25 [b]	−	2.24	5.06	0.19	a
1 e	$(CH_3)_3C$	H	H	H	4.70 [b]	+	1.98	4.72	−0.02	a
1 f	$(CH_3)_3C$	H	$(CH_3)_3C$	H	5.06 [b]	+	3.96	6.98 [c]	−1.92	a
1 g	$(CH_3)_3C$	H	H	$(CH_3)_3C$	5.52	−	3.96	6.98 [c]	−1.46	a
2. Alkyl- and halogensubstituted 1,4-benzoquinones										
2 a	Br	C_6H_5	Br	C_6H_5	4.82	+	5.64	5.19	−0.37	a
2 b	Br	CH_3	Br	CH_3	5.90	+	2.84	5.84	−0.06	[20]
2 c	Br	Br	Br	CH_3	5.65	+	3.14	6.20	−0.55	[21]
2 d	Br	Br	Br	$CH_3(CH_2)_5$	6.57	+	5.58	5.33 [c]	1.24	a
2 e	Br	CH_3	H	$(CH_3)_2CH$	5.24	+	2.97	6.00	−0.76	[22]
2 f	J	CH_3	H	$(CH_3)_2CH$	6.05	+	3.23	6.31	−0.26	[23]
2 g	Br	CH_3	Br	$(CH_3)_2CH$	7.52	+	3.83	6.90	0.62	[1,22]
2 h	N_3	CH_3	N_3	$(CH_3)_2CH$	6.85	+	3.03	6.07	0.78	[24]
2 i	Br	CH_3	$(CH_3)_2CH$	Br	7.11	+	3.83	6.90	0.21	a
2 j	Br	$(CH_3)_2CH$	$(CH_3)_2CH$	H	6.66	+	3.96	6.98	−0.32	a
2 k	Br	$(CH_3)_2CH$	$(CH_3)_2CH$	Br	7.15	+	4.82	6.75	0.40	a
3. tert-butyl- and halogensubstituted 1,4-benzoquinones										
3 a	Br	H	$(CH_3)_3C$	H	6.18	+	2.72	5.69	0.49	a
3 b	Cl	Cl	$(CH_3)_3C$	H	6.88	+	3.40	6.50	0.38	a
3 c	Br	Br	$(CH_3)_3C$	H	7.24	+	3.70	6.80	0.44	[16]
3 d	J	J	$(CH_3)_3C$	H	7.52	+	4.22	7.08	0.44	[16]
3 e	J	OH	$(CH_3)_3C$	H	5.02	−	2.43	5.31	−0.29	a
3 f	Cl	$(CH_3)_3C$	H	$(CH_3)_3C$	6.00	+	4.67	6.91	−0.91	[25]
3 g	Cl	$(CH_3)_3C$	Cl	$(CH_3)_3C$	6.34	+	5.40	5.74	0.60	[25]
4. Halogenated 1,4-benzoquinones with no directly attached alkylsubstituent										
4 a	F	F	F	F	4.45	− [e]	0.56	2.89 [c]	1.56	a
4 b	Cl	Cl	Cl	Cl	4.90	− [e]	2.84	5.84	−0.94	a
4 c	Br	Br	Br	Br	6.34	− [e]	3.44	6.54	−0.20	a
4 d	J	J	J	J	6.70	+ [e]	4.48	7.04	−0.34	[26]
4 e	Cl	N_3	Cl	N_3	5.30	−	2.34	5.20	0.10	[27]
4 f	Br	$(CH_3)_2CHO$	Br	$(CH_3)_2CHO$	5.22	+	3.78	6.86 [c]	−1.64	a
4 g	Br	$(CH_3)_2CHNH$	Br	$(CH_3)_2CHNH$	5 [d]		3.10	6.17		a
4 h	Br	$C_6H_{11}NH$	Br	$C_6H_{11}NH$	5 [d]		5.56	3.38		a

[a] See Materials and Methods.
[b] Tested as the hydroquinone.
[c] This compound has been omitted in calculations.
[d] Exact determination of pI_{50}-values was not possible because of low solubility.
[e] TMPD-bypass tested with the hydroquinones.

pI_{50}-values are listed. These values will be needed for a quantitative structure activity relationship, which will be discussed later on. As can be seen from the pI_{50}-values in Table 1.1, the inhibitory activity is increased the more and the bulkier alkyl substituents are at the quinone moiety.

Characteristic for an inhibitor of the DBMIB type is the TMPD bypass, *i. e.* the inhibition of electron flow at the plastoquinone site can be overcome by

TMPD which reconnects the two photosystems and restores electron flow. Trimethyl-(**1 c**), tetramethyl-(**1 d**), and 2,6-di-*tert*-butyl-1,4-benzoquinone (**1 g**) do not have a TMPD bypass, which might indicate that their site of inhibition is closer to photosystem II.

2. *Influence of the halogen substitution on inhibitory acitivity*

Halogensubstitution of the alkyl-1,4-benzoquinones from Table I.1 in general leads to a drastic increase in inhibitory activity (Table I, 2 and I,3). DBMIB (**2 g**) stands out by its exceptional high pI_{50}-value of 7.52. A branched alkyl side chain at the quinone moiety yields compounds with high activity. Already the pL_{50}-values of Table I.1 indicated high inhibitory potency of the parent compound.

The dimethyl-(**2 b**) and the diphenyl-derivative (**2 a**) show much smaller activity as compared to DBMIB (**2 g**), as do the tri-bromo-derivatives **2 c** and **2 d**, which bear a methyl or a *n*-hexyl group. Replacement of a methyl by a isopropyl group in DBMIB (**2 g**) does not lead to an increase in activity, in the contrary, **2 k** is somewhat less active then DBMIB. The pattern of substitution seems to be of some though small relevance. The two isomers **2 g** and **2 i** differ by 0.4 in their pI_{50}-values, the same is true for the two isomeric di-*tert*-butyl-1,4-benzoquinones **1 f** and **1 g**. A *tert*-butyl-substitutent also renders very active inhibitors (Table I.3, compounds **3 a** – **3 d**).

A bulky alkyl substitutent, however, has to be directly attached to a carbon atom of the quinone. If it is linked via a ether or amine linkage, a quinone with only poor inhibitory activity results (Table I.4, compounds **4 f** – **4 h**).

Like the alkyl substituent, the nature and number of halogen atoms at the quinone play an important role for inhibitory activity. As can be seen from Table I.1, a thymoquinone, substituted by one bromine only (**2 e**), is a relatively poor inhibitor; introduction of a second bromine (to give DBMIB **2 g**) increases activity more than hundredfold. The same increase in activity is observed in case of the *tert*-butyl-benzoquinones (Table I.2), where the monobromo compound **3 a** is less active than the corresponding dibromo compound **3 c**. The same is true for the monochloro compound **3 f** and the dichloro compound **3 g**.

Furthermore, there exists a dependency of activity on the nature of the halogen. As can be seen in Table I.2, the bromoquinone **2 e** shows less activity then the iodoquinone **2 f**. An increase in atomic weight of the halogen generally causes an increase in inhibitory power. This can be seen by comparing the chloro-, bromo- and iodoquinones **3 b**, **3 c**, and **3 d** in Table I.3 and the different tetrahalogenoquinones **4 a** to **4 d** in Table I.4. Tetrabromo-(**4 c**) and tetraiodo-1,4-benzoquinone (**4 d**) are relatively good inhibitors. A halogen apparently might replace a branched alkyl side chain necessary for high activity.

2,3-Diiodo-5-*tert*-butyl-1,4-benzoquinone (**4 d**) is an as potent inhibitor as DBMIB (**2 g**). Replacement of one iodine in **4 d** by a hydroxyl group to yield **3 e** causes a tremendous drop of the pI_{50}-value from 7.52 to a value of 5.14.

Instead of halogen, introduction of the pseudohalogen azide leads to molecules of inhibitory activity as well. Whereas **4 c** is a moderate inhibitor, the azide **2 h**, derived from DBMIB, turns out to be very active. Azides are of special interest in biochemical studies because of their use as photoaffinity labels. The azide **2 h** indeed is extremely light-labile [35], but unfortunately instead of binding to the target it is rearranging to a 1,3-cyclo-

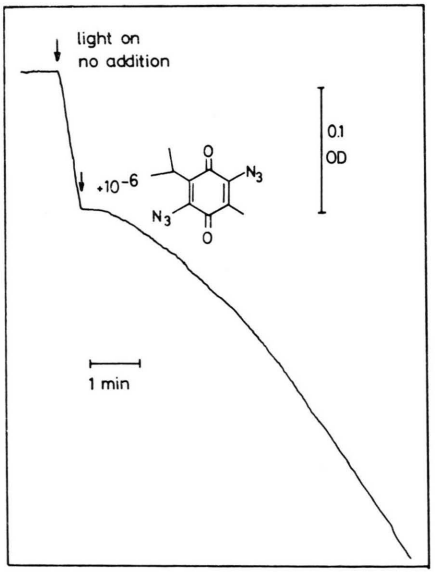


Fig. 2. Light-inactivation of 2,5-diazido-3-methyl-6-isopropyl-1,4-benzoquinone. Conditions for photosynthetic $NADP^+$ reduction are as described in Materials and Methods. Downward slope means increase in optical density.

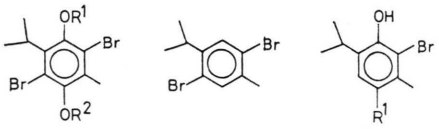
pentenedione [24]. This compound is no longer an inhibitor of photosynthetic electron transport, as can be seen from Fig. 2. After addition of 10^{-6} M of the azide, the initial rate of NADP-reduction is almost completely inhibited. Simultaneously in the light, the rearrangement reaction starts. This leads to a lowering of the concentration of the azide and in consequence to an ever increasing rate of $NADP^+$ reduction.

With the exception of 3-iodo-2-hydroxy-5-*tert*-butyl-1,4-benzoquinone (**3 e**) and the tetrahalogen-1,4-benzoquinones **4 a** – **4 c** and **4 e** all compounds tested have a TMPD bypass and therefore behave in their inhibitory pattern like DBMIB. Since the redox potentials of tetrahalogen-1,4-benzoquinones are higher than those of alkyl substituted 1,4-benzoquinones [36], chemical oxidation of TMPD ($E_0 = +270$ mV [37]) might occur. The bypass, therefore, has been tested with the hydroquinones. For the compounds with no TMPD bypass an inhibition site closer to the reducing site of photosystem II is proposed.

3. Other influences on inhibitory activity

It makes no difference in inhibition of electron transport, if DBMIB (**2 g**) or its reduced form (hydroquinone) **5 a** (Table II) are used. Both exhibit identical pI_{50}-values of 7.52. The hydroquinone might even be the active species, since it is known that chloroplasts in the light reduce quinones [32, 33] or an equilibrium between reduced and oxidized from might exist. If in the hydroquinone both hydroxyl-groups are methylated (**5 b**), so that the oxidation to the quinone cannot take place, a drastic decrease in activity is observed. Therefore, as an additional feature for an active inhibitor, the quinone/hydroquinone moiety is essential. Dibromocymene **5 c** and the mono- and dibromo-thymol (**5 d** and **5 e**) are very poor inhibitors. Also their inhibition site has shifted from the DBMIB to the DCMU type, as judged from the missing TMPD bypass.

Table II. Experimental pI_{50}-values of some compounds related to DBMIB.



5a-b 5c 5d-e

No.	R^1	R^2	pI_{50}	TMPD bypass	Synthesis Ref.
5 a	H	H	7.52	+	[18]
5 b	CH_3	CH_3	3.90	–	a
5 c	–	–	4.71	–	[28]
5 d	H	–	4.72	–	[29]
5 e	Br	–	4.77	–	[30]

[a] See Materials and Methods

Discussion

In order to get insight into the structural element necessary for inhibitory activity, structural aspects will be first discussed in a qualitative manner. The results indicate the following qualitative structure activity relationship features required for an inhibitor of the DBMIB type. A 1,4-benzoquinone with one or more bulky alkyl substitutents already yields an active inhibitor. Introduction of one or more halogen substitutents increases activity drastically as compared to the parent alkyl compound. 1,4-benzoquinones substituted by two halogens are more active than those with only one and iodination is better than bromination which again is better than chlorination.

A quantitative structure activity relationship (QSAR) is possible when sufficient data and enough compounds are available and the site of inhibition for all compounds is identical. QSAR plays an important role for biologically active molecules, especially in drug design [38]. QSAR so far has been successfully applied for several groups of inhibitors of photosynthetic electron transport like substituted phenylamides [39, 40], phenylcarbamates [41], phenylureas [41], anilides [41], imidazoles [42, 43], and triaziones [44]. A sufficient quantitative correlation was obtained by using parameters for lipophilicity, like the partition coefficient P [44], the acidity (pK_a) of the compounds under investigation [39], or both together [42]. Additional parameters for electronic and steric effects seem to be of little relevance in the case of photosynthesis inhibitors so far investigated. QSAR of such a kind in simple cases can be expressed by a linear relation of the Hansch type [40] (Eqn (1))

$$\log \frac{1}{C} \triangleq pI_{50} = a \cdot \log P + b \qquad (1)$$

where C is the concentration of inhibitor, yielding a defined response, P is the partition coefficient

and a and b are constants. If P covers more than two or three decades, in general biological activity reaches a maximum at a certain lipophilicity P_0. Compounds with lower or higher lipophilicity are less active. This non-linear relation-ship can be expressed by a parabolic Hansch equation [40] (Eqn (2))

$$\log \frac{1}{C} \triangleq \mathrm{pI}_{50} = a \cdot \log P + b \cdot (\log P)^2 + c \,. \quad (2)$$

This equation has turned out to be particularly useful [38]. However, there exists a discrepancy between (1) and (2), because (1) always yields a straight line, whereas (2) yields a parabola. In order to account for the actual circumstances, the ascending and descending part of the parabola should be linear. This approach has been realized by the so-called "bilinear model" of Kubinyi [45], which is represented by the following equation (Eqn (3))

$$\log \frac{1}{C} \triangleq \mathrm{pI}_{50} = a \cdot \log P - b \cdot \log(\beta P + 1) + c \,. \quad (3)$$

In (3), a, b and c are again constants, which can be estimated by linear multiple regression. β, the volume ratio of total lipid and aqueous phase of the biological system under investigation (V_L/V_A) is non linear and must be determined by an iteration procedure [47]. Kubinyi has shown that his model is superior to the parabolic Hansch approach, provided enough data points are available and P varies over a wide range.

For photosynthesis inhibitors it has been demonstrated so far that lipophilicity is the most important parameter in a homologous series for QSAR [38–46]. Therefore, a QSAR for the lipophilicity parameter of the quinones has been attempted. Lipophilicity parameters, as expressed by the partition coefficient P have not been experimentally determined, but calculated by summation of the hydrophobic parameters π of the substitutents in positions 2, 3, 5 and 6 of each quinone. The π-values are listed in Table III. These parameters have been determined in experiments with monosubstituted benzenes [48]. As can be seen from Table I, 1–4, the hydrophobic parameters $\Sigma \pi_{2,3,5,6}$ covered a range from 0.56 (tetrafluoro-1,4-benzoquinone **4a**) to 5.64 (2,5-dibromo-3,6-diphenyl-1,4-benzoquinone **2a**). For this reason a calculation according to (2) (parabolic Hansch approach) and (3) (bilinear model by Kubinyi) was made and both compared.

Table III. Hydrophobic (π) and electronic (σ_p) parameters for substituents [a].

Substituent	π	σ
H	0	0
CH_3	0.56	−0.17
$(CH_3)_2CH$	1.53	−0.15
$(CH_3)_3C$	1.98	−0.20
$CH_3(CH_2)_5$	3.00 [b]	−0.15 [b]
C_6H_5	1.96	−0.01
F	0.14	0.06
Cl	0.71	0.23
Br	0.86	0.23
J	1.12	0.18
OH	−0.67	−0.37
$(CH_3)_2CHO$	1.03 [b]	−0.45 [c]
N_3	0.46	0.15
$C_6H_{11}NH$	1.92 [b]	−0.61 [b]
$(CH_3)CHNH$	1.69 [b]	−0.56 [b]

[a] From Hansch *et al.* [49] if not otherwise noted.
[b] Calculated according to Leo *et al.* [50].
[c] From Fujita and Nishioka [51].

Parabolic Hansch approach (Eqn (4))

$$\mathrm{pI}_{50} = 4.03\,(\pm 1.48)\,\Sigma\pi - 0.49\,(\pm 0.20)\,(\Sigma\pi)^2 - 1.44 \,. \quad (4)$$

Bilinear model Kubinyi (Eqn (5))

$$\mathrm{pI}_{50} = 1.33\,(\pm 0.36)\,\Sigma\pi - 4.04\,(\pm 1.34)\,\log(10^{\Sigma\pi}\cdot\beta + 1) + 2.10 \,, \quad (5)$$

	Hansch	Kubinyi
N	25	25
R^2	0.648	0.725
s	0.57	0.50
F_{test}	19.4	27.7
P_0	4.11	4.28

where N represents the number of compounds, R^2 the coefficient of multiple determination, s the standard deviation and F the value of the F-test for null hypothesis $a = b = c = 0$. Values in parantheses are the 95% confidence intervals.

From 34 quinones only 25 have been included in the calculations. Thymoquinone (**1a**), 2-*n*-hexyl- (**1b**), 2,5-dibromo-3,6-di(isopropylamino)- (**4g**) and 2,5-dibromo-3-6-di(cyclohexylamino)-1,4-benzoquinone (**4h**) have been excluded, because their pI_{50}-values because of low solubility could not be determined exactly. The two isomeric di-*tert*-butyl-benzoquinones **1f** and **1g**, 2,3,5-tribromo-6-*n*-hexyl- (**2d**), tetrafluoro- (**4a**), and 2,5-dibromo-3,6-di(isopropyl-oxy-1,4-benzoquinone (**4f**) have been omitted, because $\Delta\,\mathrm{pI}_{50} > 1.2$.

As can be seen from the comparison of the R^2 and F-values for both models, the Kubinyi model gives a significant better fit. A plot of lipophilicity versus pI_{50} is shown in Fig. 3. As already stressed, besides lipophilicity other parameters can be taken in consideration for QSAR. Eqn (5) could not be improved by including other parameters like $\Sigma\sigma_p$ (electronic effects) or Σ_{MR} (molecular refraction; measure of steric bulk). The residuals (ΔpI_{50}) according to (5) are not normally distributed, as one might expect. In contrast, there seems to be a systematic dependence of the residuals from $\Sigma\sigma_p$. Quinones with only one halogen substitutent have negative residuals, *i. e.* $\Delta pI_{50} < 0$ (with the exception of 2-bromo-5-*tert*-butyl-1,4-benzoquinone (**3 a**)), whereas quinones with two halogen substitutents have positive residuals, *i. e.* $\Delta pI_{50} > 0$ (with the exception of 2,5-dibromo-3,6-diphenyl-1,4-benzoquinone (**2 a**)). A comparison with the corresponding $\Sigma\sigma_p$-values shows that negative residuals correlate with $\Sigma\sigma_p < 0$ and positive residuals with $\Sigma\sigma_p > 0$. This correlation could not be expressed by a simple linear relationship and for this reason not be included into Eqn (5). A qualitative estimation suggests that the variation in $\Sigma\sigma_p$, which was not taken into account, might explain for variation in calculated pI_{50}-values up to 0.3. Besides the electronic effect there definitely exists an effect of the position of the substitutent. pI_{50}-values in the two isomeric dibromo-methyl-isopropyl-benzoquinones **2 g** and **2 i** and the isomeric di-*tert*-butyl-benzoquinones **1 f** and **1 g** differ in between 0.4 to 0.5. Because there were only these two couples available, a further evaluation was not possible. Taking electronic and position effects together, there exists a possible variation range in pI_{50}-values of 0.5 – 0.8, which is not accounted for by (5). This value even exceeds the standard deviation of 0.5

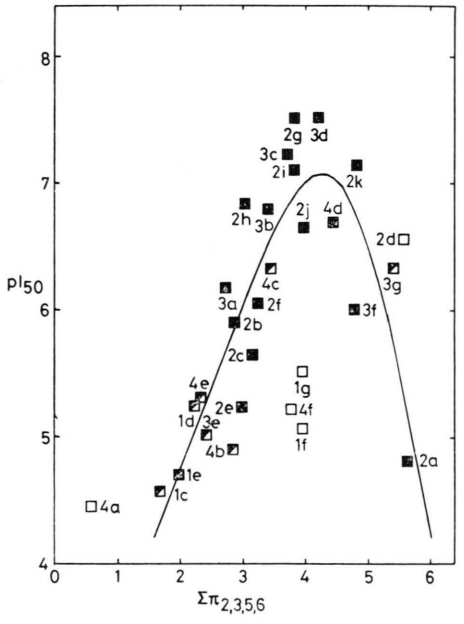


Fig. 3. Relationship between pI_{50} and hydrophobic parameter $\Sigma\pi_{2,3,5,6}$ for 1,4-benzoquinones according to the bilinear model ■ TMPD bypass, ◪ no TMPD bypass, □ omitted in calculation.

A value of 0.72 for R^2, *i. e.* that 72% of the variation in pI_{50}-values of the quinones are explained by (5) does not seem very high. One has to take into account, however, that electronic parameter and parameter of position, which obviously play a role for QSAR of benzoquinones have been disregarded in Eqn (5) and that Eqn (5) applies for all 1,4-benzoquinones, regardless the number and nature of substitutents. Taking this considerations into account, the value of 0.72 for the correlation factor does not look too bad.

As already pointed out, some of the quinones tested do not exhibit a TMPD bypass. This might indicate an inhibition site closer to the reducing site of photosystem II, as already shown for some other quinones [6]. Surprisingly, these quinones nevertheless fit well into the QSAR. This might lead to the conclusion, that although the inhibition sites are different the lipophilicity requirements for both sites are very similar.

We are, however, as yet unable to explain why a few quinones differ in more than 1.2 from the calculated pI_{50}-values. In case of tetrafluoro-1,4-benzoquinone **4 a**, which was the first fluorinated quinone so far tested, the unique role of fluorine as a strong electron with-drawing substitutent might account for the deviation. No explanation so far could be found for the exceptional low pI_{50}-values of the di-*tert*-butyl-1,4-benzoquinones **1 f** and **1 g** and the 2,5-dibromo-3,6-di(isopropyloxy)-1,4-benzoquinone **4 f**.

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