The Effect of Analogues of Dibromothymoquinone and of Bromonitrothymol on Photosynthetic Electron Flow

A. Trebst and S. Reimer

Lehrstuhl für Biochemie der Pflanzen, Ruhr-Universität, Postfach 102148, D-4630 Bochum 1

W. Draber and H. J. Knops

Bayer AG, Forschungszentrum, Wuppertal

Z. Naturforsch. **34 c,** 831 – 840 (1979); received June 1, 1979

Photosynthesis, Inhibitors, Herbicides, Dibromothymoquinone, Nitrophenols, Chloroplast

Alkyl substituted derivatives of halogenated *p*-benzoquinones, of halogenated *p*-nitrophenols, and of 2,4-dinitrophenols were tested in the inhibition of photosynthetic electron flow in chloroplasts. The effect of the compounds on photoreductions by photosystem I or II, on a TMPD bypass in NADPH formation and the reversibility of the inhibition by dithiothreitol is used to distinguish between an inhibition site before or after plastoquinone function, *i. e.* between a DBMIB versus a DCMU inhibition pattern.

It is shown, that different isopropyl and *t*-butyl substituted halogenated *p*-benzoquinones are as effective and specific as DBMIB in the inhibition of plastoquinone function.

Alkyl substituted *p*-nitrophenols, with an additional halogen- or nitro-group at C-2, are shown to be effective electron flow inhibitors. The new potent nitrophenol derivatives inhibit at the site of DCMU action, nevertheless they do not contain the basic chemical element essential for inhibition common to DCMU and its many herbicidal analogues. Small changes in the ring-substitution can alter the inhibition pattern from a DCMU typ to a DBMIB typ inhibition.

Introduction

Numerous compounds are inhibitors of photosynthetic electron flow at the level of the primary quencher of photosystem II and before the reduction of plastoquinone. Among these inhibitors, derived chemically from substituted ureas, anilides, triazines, uraciles, pyridazinones, triazinones and others, are many commercial herbicides (for review see [1 to 4]). This group is best represented by DCMU-diuron, because this compound is used most commonly in photosynthesis research. The relation of chemical structure to biological (inhibitory) activity of such inhibitors has been widely studied. Inhibition values in photosynthetic reactions in isolated chloroplasts are preferentially used in such studies, because of their relatively exact nature. The basic structural chemical element in the compounds, essential and responsible for inhibition of function or for binding to the inhibition site has been elucidated [1 – 4] to be the sp^2 hybrid $-\overset{\|}{C}-\bar{N}-$.

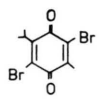
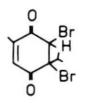
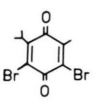
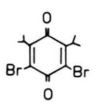
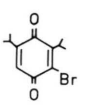
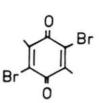
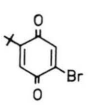
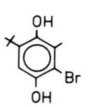
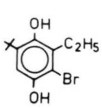
Abbreviations: DBMIB, 2,5-dibromothymoquinone; DCMU, 3-(dichlorophenyl) 1,1-dimethylurea; Dinoseb, 2,4-dinitro-6-*sec*-butylphenol; DNP, 2,4-dinitrophenol; DTT, dithiothreitol; LIS, lithium salt of diiodosalicyclic acid; PD, *p*-phenylenediamine; PS I or II, photosystem I or II; TMPD, N-tetramethyl-*p*-phenylenediamine.

Reprint requests to Prof. Dr. A. Trebst.

0341-0382/79/0900-0831 $ 01.00/0

With the introduction of the halogenated alkyl substituted benzoquinone DBMIB as a plastoquinone antagonist, a new type of inhibitor of photosynthetic electron flow became available [5]. The inhibition site for DBMIB is *after* the functional site of plastoquinone, *i. e.* at the reoxidation of plastoquinol and therefore different from that of the DCMU type inhibitor, which inhibits *before* the main pool of plastoquinone function. The use of DBMIB made possible, in particular, the measurement of photoreductions by photosystem II only. Numerous papers on this have been published by now from many laboratories (for review see [6, 7]).

In addition to DBMIB some other substituted benzoquinones or inhibitors of photosynthetic electron flow have been reported [8 – 10], but not yet chemical structure/biological activity relationships on this group of inhibitors. This paper describes some new DBMIB analogues and their inhibition of photosynthesis. Furthermore, we wish to show that, and which, changes in chemical substitutents shift the inhibition point of halogenated alkyl substituted benzene derivatives in the photosynthetic electron transport chain from that of DBMIB to that of DCMU. New very effective inhibitors of photosynthetic electron flow at the DCMU site – alkylsubstituted halogenated nitrophenols or dinitrophenols – are described. Though these compounds show inhibition

					Rate of electron flow in the presence of inhibitor [μ moles / mg chlorophyll · h]					
					Photoreduction of FeCy		Photoreduction of $NADP^+$			
		I_{50}	pI_{50}	inhibitor conc.	−PD	+PD	−TMPD	+TMPD	+DTT	comments
	control without inhibitor				180	252	129	123	126	
1	 DBMIB	$2 \cdot 10^{-7}$	6.7	10^{-6} 10^{-5}	43 55	180 79	20 <5	126 95	120 96	changes inhibition pattern at high conc.
2		$5 \cdot 10^{-5}$	4.3	$5 \cdot 10^{-5}$	177	166	72	108	118	acceptor of PS II
3		$3 \cdot 10^{-7}$	6.55	$5 \cdot 10^{-6}$	126	202	<5	113		acceptor of PS II, is oxidized to DBMIB
4	 Iso-DBMIB	$2 \cdot 10^{-7}$	6.7	$2 \cdot 10^{-6}$	87	228	11	111	105	
5		$2 \cdot 10^{-7}$	6.7	10^{-6}	78	205	12	118	89	
6		10^{-6}	6	$5 \cdot 10^{-6}$	138	198	8	126	96	
7		$5 \cdot 10^{-5}$	4.3	10^{-5} $5 \cdot 10^{-5}$	109 61	165 69	57 <5	113 53	122 107	acceptor of PS I cyclic ATP
8		$5 \cdot 10^{-6}$	5.3	10^{-5}	145	224	21	127	45	
9		$2 \cdot 10^{-5}$	4.7	$2 \cdot 10^{-5}$			57	59	59	acceptor of PS I cyclic ATP
10		$4 \cdot 10^{-5}$	4.4	$5 \cdot 10^{-5}$	127	180	69	129		

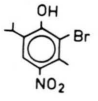
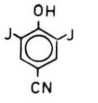
11		$5 \cdot 10^{-5}$	4.3	10^{-4}	92	125	61	81	102	acceptor of PS I cyclic ATP
12		10^{-4}	4.0	$2 \cdot 10^{-4}$	73	70	29	29		acceptor of PS I cyclic ATP
13		$2 \cdot 10^{-7}$	6.7	10^{-6}	13	19	21	17	21	
14		$2 \cdot 10^{-7}$	6.7	$2 \cdot 10^{-7}$ $5 \cdot 10^{-7}$	65 29	110 20	60 19	85 22	59 19	

Table I. Inhibition of photosynthetic electron flow by halogenated alkyl substituted benzoquinones. The ferricyanide Hill-reaction in the presence or absence of 0.1 mM *p*-phenylenediamine was measured as oxygen evolution, $NADP^+$ reduction in the presence or absence of 0.1 mM TMPD or of 0.1 mM DTT by absorption change at 340 nm as well as by oxygen evolution.

patterns like DCMU, they obviously contain another essential basic chemical element, different from the one in the well known inhibitors of the DCMU type. Among this new inhibitors are already known herbicidal but also new very potent representatives of the dinitrophenol type.

Materials and Methods

Washed chloroplast thylakoids were prepared from spinach leaves according to Nelson *et al.* [11]. Photosynthetic activity was measured at 18 °C with 35,000 lx white light or 2.5×10^5 erg./cm^{-2} sec^{-1} red light. The medium contained in a volume of 3 ml (µmol): tricine-NaOH, pH 8.0, 80; $MgCl_2$, 10; ADP, 10; inorganic phosphate 10; and as electron acceptors either ferricyanide, 20; or $NADP^+$, 6, plus ferredoxin, 0.01, and chloroplasts with a chlorophyll content of 200 µg or 20 µg respectively. Oxygen evolution was followed manometrically, NADPH formation was determined at 340 nm. pI_{50} values are measured in the presence of an uncoupler and are not corrected for zero chlorophyll concentration.

Of the inhibitors, some of the dinitrophenols may be obtained from Ferak Berlin, 1000 Berlin 47. The others have been synthesized at Bayer AG, Forschungszentrum Wuppertal.

Results

The different biochemical characteristics of the influence on photosynthetic electron flow by a DCMU type *versus* a DBMIB type inhibitor may easily be observed in isolated chloroplasts. In intact thylakoid membranes of chloroplasts DCMU inhibits the photoreduction of all Hill-acceptors (except silicomolybdate), whereas DBMIB inhibits only the photoreduction of photosystem I acceptors, like $NADP^+$ or methylviologen with water as the electron donor. The DBMIB insensitive, but DCMU sensitive, photoreduction of Hill-acceptors by photosystem II only is best measured by a ferricyanide reduction, mediated by a lipophilic phenylenediamine (see [6, 7]). In the absence of phenylenediamine a certain rate of ferricyanide reduction remains in the presence of DBMIB, reflecting the (in-)accessibility of the reducing end of PS II to a hydrophilic acceptor. The complete inhibition by DBMIB of $NADP^+$ reduction and of oxygen evolution which require both PS I and PS II, may be reversed by a thiol compound [13, 14] (like DTT). In the first case, an internal electron flow bypass via TMPD occurs, in which electrons are accepted at photosystem II by oxidized TMPD before the DBMIB block and donated back by reduced TMPD into photosystem I after the block [12], see scheme in Fig. 2. A thiol compound on the

other hand adds onto quinones and thus inactives the inhibitor [13, 14]. The five reaction types: a) ferricyanide reduction by PS I, b) ferricyanide reduction by PS II (*i. e.* in the presence of phenylenediamine), c) $NADP^+$ reduction, d) TMPD bypass in $NADP^+$ reduction, e) thiol effect in the $NADP^+$ system, have been used for characterisation of the inhibition pattern of the new inhibitors, presented here. DCMU will inhibit all these reactions, DBMIB only reaction a and c.

In Table I are compiled these biochemical properties of a number of possible DBMIB analogues. The concentration which inhibits photosynthetic $NADP^+$ reduction to 50% is indicated as I_{50} as well as the negative logarithm of it (pI_{50} value), which is usually used for comparison of chemical parameters according to a Hammet equation. The next column shows photosynthetic activities (at the concentration of inhibitor indicated) in a ferricyanide Hill-reaction ± PD and a $NADP^+$ reduction ± TMPD or the thiol DTT. Accordingly DBMIB at 1 μM inhibits ferricyanide reduction by PS I, but not by PS II (*i. e.* in the presence of PD). $NADP^+$ reduction is completely inhibited, but restored by a TMPD bypass, as well as by the addition of a thiol like DTT. At 10 μM DBMIB photosystem II reductions are also impaired to some extent. The Table I then shows that other alkyl substituted brominated *p*-benzoquinones, like "iso-DBMIB" (substance **4**) or dibromo-di-isopropyl-benzoquinone (substance **5**) are as effective inhibitor as is DBMIB. Monobromothymoquinone (substance **2**) on the other hand is a poor inhibitor, the monobromo derivative **6** of intermediate inhibitory activity. The compound **2** as well as the intermediate in DBMIB systems, (the dibromo adduct onto thymoquinone, substance **3**) are highly autoxidable compounds, which act also as acceptor of PS II. The intermediate is slowly oxidized to DBMIB changing its inhibitory pattern.

Dibromo-dimethyl-benzoquinone (substance **7**) shows a different behaviour than DBMIB, as do also the two dichloro-benzoquinones (substances **11** and **12**), because they do not support a phenylene-diamine mediated photosystem II photoreduction. Their inhibition of $NADP^+$ reduction is still reversed by DTT (they are quinones), but the TMPD bypass is only weakly active. The inhibition pattern by these compounds corresponds very much to high concentrations (10^{-5} M) of DBMIB, as indicated in the Table. As already observed, at this concentration DBMIB also inhibits photoreductions by PS II and the P/e_2 ratio falls [12], indicating a shift of the inhibition site toward photosystem II. This shift has been observed with other quinone antagonists as well [10].

In a number of cases the P/e_2 ratio of an impaired non-cyclic system actually rises, upon addition of inhibitor. This is due to an overimposed coupled cyclic electron flow around PS I. This is indicated in Table I in "comments" (substances **7**, **9**, **11**, **12**). A cyclic electron flow around PS I also results in an inhibition of non-cyclic $NADP^+$ reduction. This type of inhibition should not be confused with the DBMIB type of inhibition, though at first glance they look rather similar.

In sum, dibromo- and alkyl-substituted benzoquinones are inhibitors of electron flow specifically as plastoquinone antagonists at its oxidation site analogues to DBMIB. Simple benzoquinones show less specifity and other activities may superimpose.

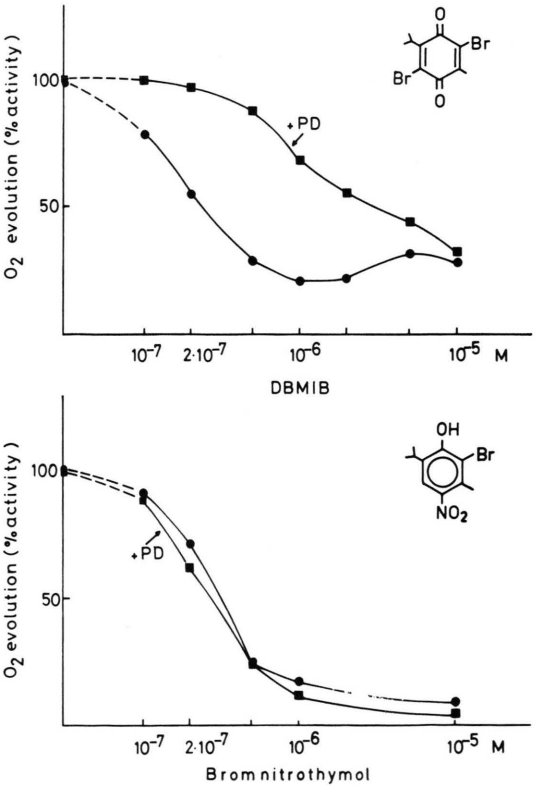


Fig. 1. The different effect of two structurally related inhibitors (DBMIB and bromonitrothymol) on the photoreduction of ferricyanide in the absence or presence of 0.1 mM *p*-phenylenediamine.

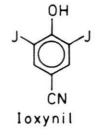
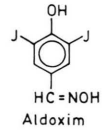
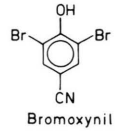
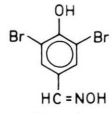
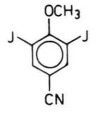
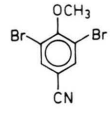
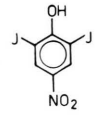
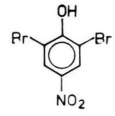
			Rate of electron flow in the presence of inhibitor					
				Photoreduction of				
				FeCy		$NADP^+$		
				[μ moles / mg chlorophyll · h]				
	I_{50}	pI_{50}	inhibitor conc.	−PD	+PD	−TMPD	+TMPD	+DTT	comments
control without inhibitor				180	252	129	123	126	
 Ioxynil	$2 \cdot 10^{-7}$	6.7	$5 \cdot 10^{-7}$	29	20	19	22	19	
 Aldoxim	$2 \cdot 10^{-5}$	4.7	$5 \cdot 10^{-5}$	15	60	30	112	100	ATP formation also restored
 Bromoxynil	$2 \cdot 10^{-6}$	5.7	$5 \cdot 10^{-6}$	27	45	30	30	50	
 Aldoxim	10^{-3}	3.0	10^{-3}			69	109		
	$3 \cdot 10^{-4}$	3.85							
	$7 \cdot 10^{-4}$	3.3							
		6.23							
		5.62							

Table II. Inhibition of photosynthetic electron flow by halogenated hydroxybenzonitriles compared with the corresponding banzaldoximes and nitrophenols. Conditions as in Table I.

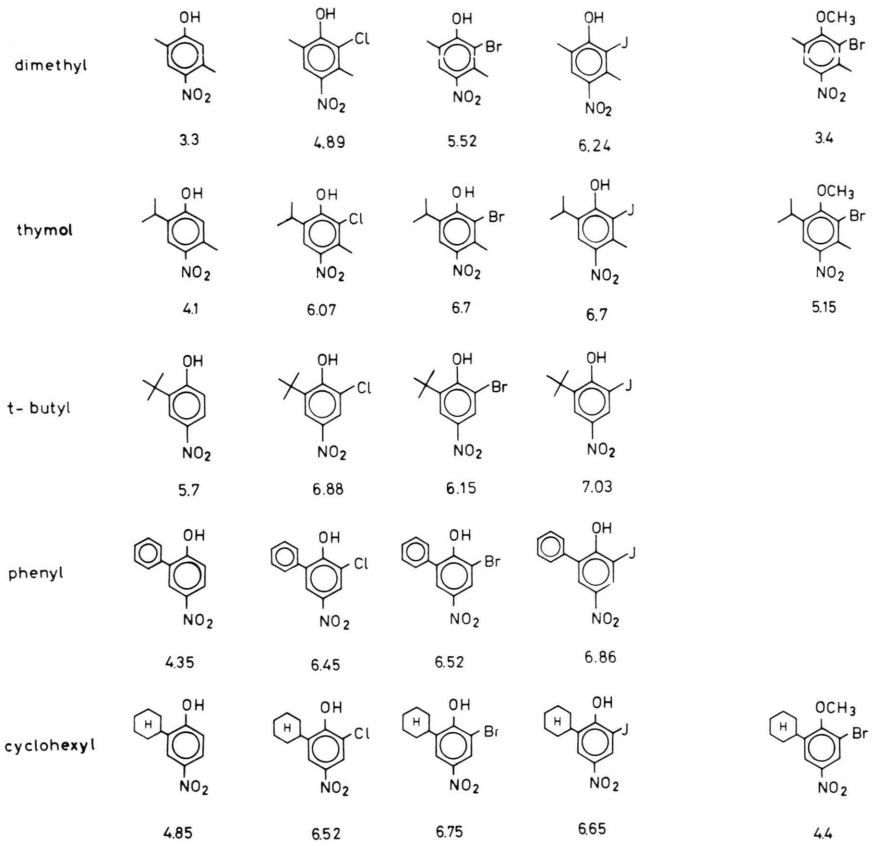


Table III. Inhibition of photosynthetic electron flow by halogenated and alkyl substituted nitrophenols. The figures are the negative logarithm of I_{50} (= pI_{50}) in $NADP^+$ reduction.

The last two compounds in Table I also show some structural relationship to DBMIB, but they are phenols and their inhibition pattern is completely different. Bromonitrothymol and ioxynil inhibit all Hill-reactios, they also inhibit the TMPD bypass and the inhibition is not reversed by DTT (they are not quinones). Indeed, these compounds have an inhibitory pattern like DCMU. The difference in inhibitory pattern of DBMIB compared with bromonitrothymol is specified in Fig. 1. In the case of DBMIB the photoreduction of ferricyanide *via* PD (*i. e.* photoreduction by PS II) is not inhibited in a certain concentration range. No complete inhibition is obtained even at very high concentrations. In the case of bromonitrothymol, however, both systems show the same sensitivity and the systems are inhibited to 100%.

In Table II the effect of ioxynil, bromoxynil and their synthesis precursors, the two benzaldoximes are compared. These nitrile herbicides are well known as photosynthesis inhibitors [15 – 18]. The oxime derivatives are much less inhibitory than the corresponding nitriles, as also already reported [19]. The difference is, however, that the benzaldoximes have an inhibition pattern somewhat like DBMIB, whereas the nitriles behave like DCMU. This is concluded from the observation, that the oxime inhibition of $NADP^+$ reduction is partly reversed by a TMPD bypass, whereas that of the nitriles is not. This indicates a shift of inhibition site from that DCMU inhibition towards (but not identical with) that of DBMIB inhibition to a point after plastoquinone function. On the other hand, the PD stimulated photosystem II photoreduction remains sensitive to the aldoximes. Methylation of the hydroxy group largely abolished inhibitory activity. The dihalogenated nitrophenols have similar properties and potencies as a dihalogenated cyanophenols.

Of the compounds in Tables I and II and Fig. 1, then, one can distinguish "true" DBMIB analogues of similar inhibitory efficiency and pattern from those of weak inhibitory activity and changed inhi-

bitory pattern, and thirdly from those compounds with high inhibitory effectiveness but completely different activity pattern.

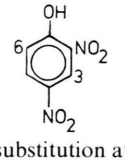
The different and very effective inhibition pattern of bromonitrothymol led us to investigate more compounds of this type. Table III lists the pI_{50} values of a number of substituted halogenated nitrophenols. All these compounds are inhibitory to photosynthetic electron flow like DCMU and unlike DBMIB, that is they inhibit all the five reactions tested in Table I.

The Table III indicates, which substituents are yielding an effective photosynthesis inhibitor: alkyl or aryl substitution and halogenation. The dimethyl substitution series yields only moderately effective inhibitors, whereas the thymol, *t*-butyl, phenyl and cyclohexyl series yield highly effective electron transport inhibitors. Methylation of the OH-group diminishes activity as it did in the case of hydroxynitriles (Table II). In general the iodo substitution yields better inhibitors than bromo- and much better than chloro-substitution. Further effective compounds and detailed structure activity relationships have recently been described [4]. The indicated pI_{50} values are valid for the chlorophyll concentration used and are not corrected for zero chlorophyll.

Instead of a halogenation, also an additional second nitro-group enhances inhibitory potency – compare 2-bromo-4-nitrothymol (Table III) with 2,4-dinitrothymol (Table IV). By this it is recognized that the weak electron flow inhibitor DNP may be converted into a highly active compound by increasing the alkyl substitution. By comparison of Tables III and IV, it is seen that halogenation is superior to a second nitro-group in increasing the pI_{50}. In the dinitrophenol series, the pI_{50} increases with the number of carbon atoms in the alkyl sidechain. From the three isomeric compounds: *sec*-butyl, *t*-butyl- and methyl-isopropyl-dinitrophenol is the *t*-butyl derivative the best inhibitor. Cyclohexyl-dinitrophenol has a pI_{50} value even above 6. It has recently been recognized, that the well known herbicides DNOC and dinoseb are more effective as inhibitors of photosynthetic electron flow than as uncoupler [3, 20 – 23]. Van Rensen *et al.* took great care to identify the inhibition site of DNOC being identical to the one of DCMU [22, 23].

The hydroxy-halogenated benzonitriles fall biochemically in the same group as the nitrophenols. Of course, these herbicides are long known to be photosynthesis inhibitors, though there inhibitory mode of action till now was a matter of debate [18 – 21]. The halogen substituted nitrophenols of Tables II and III and dinitrophenols of Table IV may now be added to this effective group of inhibitors of photosynthetic electron flow at the DCMU inhibition site.

Table IV. Inhibition of photosynthetic electron flow by substituted 2,4-dinitrophenols at the site of DCMU.



substitution at

	C-6	C-3	pI_{50}
DNP	–	–	3
DNOC	methyl	–	3.7
	methyl	methyl	4.92
Dinoseb	sec. butyl	–	5.1
Dinitrothymol	*i*-propyl	methyl	5.6
	t-butyl	–	5.78
	cyclo-hexyl	–	6.05

Discussion

DBMIB has established itself now as a very useful inhibitory compound to study the mechanisms of photosynthesis (see review [6, 7]). Other quinones which also inhibit photosynthetic electron flow had been described at the same time when DBMIB was introduced. These phythyl substituted, halogenated or hydroxylated benzoquinones [8, 9] with their long lipophilic sidechain are, however, less easily handled, than DBMIB and also are less effective. More disadvantagous is, that their inhibition site is less exactly established. It should be noted, however, that the "pure" inhibition site (1 μM) of DBMIB at the reoxidation of plastohydroquinone is also superimposed by an inhibition site at the reduction of the main pool of plastoquinone, when higher concentrations (5 μM) of DBMIB are employed [6, 7, 12] (see below).

The new DBMIB analogues, described here, are other alkyl substituted, brominated benzoquinones, as effective as DBMIB and with matching influence on the photosynthetic electron flow system. It appears that a lipophilic, though short, alkyl substitu-

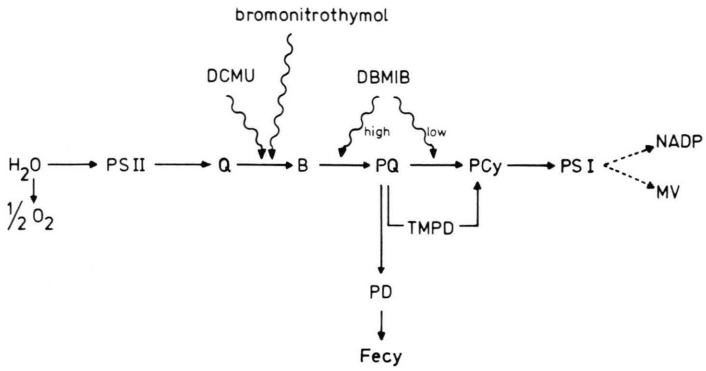


Fig. 2. Inhibition sites of DBMIB and bromonitrothymol in the area of plastoquinone function in photosynthetic electron flow. The TMPD bypass of the inhibition site between plastoquinone and plastocyanin and the PD mediated photosystem II driven FeCy photoreduction are indicated.

tion is essential but also sufficient for an effective inhibitor, isopropyl substitution being already optimal, whereas dimethyl substitution is insufficient. The position of the halogen on the ring is not of much importance, 2,5-(= DBMIB) and 3,5-dibromo (= iso DBMIB) derivatives are equally effective. Monobromo substitution is also, though less, effective. It seems that no particular substitution of the quinone ring (recall, that it has a symmetry axis) is required for inhibition. Though very probably the hydroquinone from is the actual inhibitors species [14], a redox function seems to be essential for an inhibitor of the DBMIB type. This follows from the change of the inhibition site when the ring substitution is not altered, but a phenol instead of a hydroquinone is tested. By preparing such phenols, very effective inhibitors were found, but with very different inhibitory properties (see below).

The structural relationship of bromonitrothymol to bromonitrothymoquinone is apparent. However, the two compounds have quite different sites of inhibition of the electron transport chain. The bromonitrothymol is inhibiting before, DBMIB after the function of the main pool of plastoquinone. However, as reported earlier already, also DBMIB shifts its point of inhibition in the electron flow system closer to that of DCMU when its concentration increases [12]. This is indicated by changed biochemical behaviour (inhibition of PD mediated photosystem II reductions) as well as influence on the fluorescence induction curve [24]. But the mode of action, as well as biochemical characteristics, of the inhibition by high concentrations DBMIB are still different from that of bromonitrophenol. For example the reversal of inhibition by thiol compounds and the insensitivity of the TMPD bypass to DBMIB analogues at any concentration is not possible with bromonitrophenol. Of course, a thiol reversal just reflects the quinone property of the DBMIB type inhibitors. Furthermore, bromonitrothymol replaces a radioactive labelled metribuzin (a DCMU analogue) from the thylakoid membrane [14]. This indicates – according to Tischer and Strotmann [25] – identical binding sites of bromonitrothymol, metribuzine and DCMU.

Both types of inhibitors represented by DBMIB and bromonitrophenol are effective in the region of plastoquinone function. It becomes accepted now, that the primary quencher of photosystem II (Q or X_{320}), and the intermediate compounds B (or R) before the main plastoquinone functional pool are all particular species of plastoquinone (for review see [26]). Therefore the double action of DBMIB, inhibition before or after plastoquinone function, depending on concentration, might not be surprising. At low concentration it just effects plastoquinone oxidation, at higher concentration also its reduction (see scheme in Fig. 2).

Phenols, like bromonitro- or dinitro-thymol – structurally related to the bromo-thymoquinone – shift their inhibition point even further to the primary plastoquinone acceptor species (Fig. 2). Their biochemical inhibition pattern is alike that of DCMU. Also diiodo-nitrophenol and diiodo-benzonitril (ioxynil) belong to this group. Indeed, ioxynil is able to replace a labelled DCMU analogue (metribuzin) from the binding site on the membrane [14]. The aldoximes, corresponding to ioxynil and bromoxynil, however, have some tendencies to behave like high concentrations of DBMIB, without implying at all that the binding sites are identical. The highly effective inhibition of photosynthetic electron flow by ioxynil has long been recognized [15 – 18].

DNP, the principle uncoupler of respiration had been found to be little effective in photophosphory-

lation, but a slight effect on electron flow inhibition had been reported [27 – 29]. Siow and Unrau [20] and more recently Moreland *et al.* [3, 21] recognized that dinitrophenols with some alkyl sidechain are electron flow inhibitors together with some uncoupling activity in photosynthesis. They grouped such compounds as inhibitory uncouplers [3]. Van Rensen *et al.* showed carefully that dinitrocresol (DNOC) is a specific electron flow inhibitor at the DCMU inhibition site [22, 23]. The halogenated nitrophenols and dinitrophenols with longer alkyl sidechains like thymyl, *t*-butyl, cyclohexyl and phenyl reported on here, are much more effective electron flow inhibitors than DNP or DNOC. Their uncoupling activities, though there, is low compared with the electron flow inhibition. Among these compounds are the well known herbicides DNOC and Dinoseb, whose mode of action had been thought so far to be in respiration rather than photosynthesis (see for example [2, 3, 30]). Also among the halogenated nitrophenols described here as potent inhibitors of photosynthetic electron flow, effective herbicides are known [30, 31]. Furthermore, the effective electron flow inhibitor *n*-butyl-3,5-diiodo-4-hydroxy benzoate described by Avron and Shavit [32] as well as Lis (lithium salt of diiodo-salicylat – a protein binding compound) checked on fluorescence pattern by Homann [33] belong to this new class of photosynthesis inhibitors with biochemical behaviour like bromonitrothymol and the other halogenated cyano- and nitrophenols, described here.

From studies on the relationship of chemical structure to biological activity among the many DCMU analogues it has long been recognized that the sp^2 hybrid $-\overset{\|}{C}-\bar{N}-$ is the essential basic chemical element responsible for inhibition [1 – 4]. The nitrophenols and analogues reported on here, do not contain this group, but still inhibit in the same way. Furthermore, the relationship of biological activity to chemical substitution follows quite different rules. In the DCMU and its analogue families inhibitor potency depends on lipophilic and electronic parameters [1 – 4]. The inhibitory activity of phenol inhibitors on the other hand, depends on steric parameters only [4], the best fit being obtained with the model of Verloop *et al.* [34]. Still all evidence on the biochemical site of inhibition of DCMU and bromonitrothymol, including the replacement technique of Tischer and Strotmann [25], in which a radioactive labelled inhibitor is replaceable by another chemically and biochemically related inhibitor, indicate identical binding sites for DCMU, ioxynil and bromonitrothymol in the membrane [14, 35]. It is conceivable that the binding site in the protein in the functional area of photosystem II, catalyzing electron flow from Q to plastoquinone via its prosthetic group B has several attachement groups for inhibitors in a binding area and is influenced by different substitutions and basic essential elements of different inhibitors in such a way that the binding of either one or another group is rate limiting. This has been discussed in more detail recently [4].

The relation of this B-protein to the functional elements in plastoquinone oxidation is not at all apparent. Such a relation is indicated, however, by the change of inhibition pattern by slight changes in the chemical substitution pattern of the inhibitors. As we reported recently, dinitrophenylethers of bromonitrothymol [36], of iodonitrothymol [36] and of ioxynil [35] inhibit photosynthetic electron flow clearly at the DBMIB inhibition site (see also [4]). The dinitrophenylation of the phenol herbicides has shifted the inhibition site from a site before to one after plastoquinone function [4, 35, 36]. This stresses again that slight changes in the substitution pattern of halogenated phenols and quinones shifts the inhibition site in the area of plastoquinone function between Q, B and the main PQ pool (Fig. 2), *i. e.* they interfere at different stages in the chemical mechanism of the reduction of plastoquinone *via* a semiquinone and the dianion or of the oxidation of plastoquinol. This has also been stressed by Barr *et al.* for other plastoquinone antagonists [10]. It should be mentioned that Robinson *et al.* [37] have reported that trifluralin, a dinitroaniline herbicide inhibits photosynthetic electron flow like DBMIB.

[1] K. H. Büchel, Pesticide Science **3,** 89 (1972).
[2] J. R. Corbett, The Biochemical Mode of Action of Pesticide, Academic Press, London-New York 1974.
[3] D. E. Moreland and J. L. Hilton, Herbicides, **Vol. I,** (ed. L. J. Audus), p. 493, Academic Press London-New York 1976.
[4] A. Trebst and W. Draber, Advances in Pesticide Science, **Part 2,** (ed. H. Geissbühler), p. 223 – 234, Pergamon Press, Oxford and New York 1979.
[5] A. Trebst, E. Harth, and W. Draber, Z. Naturforsch. **25 b,** 1157 (1970).

[6] A. Trebst, Proc. of the 3rd. Int. Congr. on Photosynthesis, Rehovot (ed. M. Avron), p. 439, Elsevier Scientific Publishing Com., Amsterdam 1974.
[7] S. Izawa, Encyclopedia of Plant Physiology, New Series, **Vol. V,** (ed. A. Trebst and M. Avron), p. 266, Springer Verlag, Heidelberg 1977.
[8] C. J. Arntzen, J. Neumann, and R. A. Dilley, Bioenerg. **2,** 73 (1971).
[9] J. Bolen, R. Pardini, H. T. Mustafa, K. Folkers, R. A. Dilley, and F. L. Crane, Proc. Nat. Acad. Sci. USA **69,** 3713 (1972).
[9 a] W. Oettmeier, S. Reimer, and K. Link, Z. Naturforsch. **33 c,** 695 (1978).
[10] R. Barr, F. L. Crane, G. Beyerm, L. A. Maxwell, and K. Folkers, Europ. J. Biochem. **80,** 51 (1977).
[11] N. Nelson, Z. Drechsler, and J. Neumann, J. Biol. Chem. **245,** 143 (1970).
[12] A. Trebst and S. Reimer, Z. Naturforsch. **28 c,** 710 (1973).
[13] S. Reimer and A. Trebst, Z. Naturforsch. **31 c,** 103 (1975).
[14] S. Reimer, K. Link, and A. Trebst, Z. Naturforsch. **34 c,** 419 (1979).
[15] N. W. Kerr and R. L. Wain, Ann. Appl. Biol. **54,** 447 (1964).
[16] D. Paton and J. E. Smith, Nature **207,** 1211 (1965).
[17] Z. Gromet-Elhanan, Biochem. Biophys. Res. Commun. **30,** 28 (1968).
[18] S. Katoh, Plant and Cell Physiol. **13,** 273 (1972).
[19] D. E. Moreland and W. J. Blackmon, Weed Science **18,** 419 (1970).
[20] K. S. Siow and A. M. Unrau, Biochemistry **7,** 3507 (1968).
[21] W. R. Alsop and D. E. Moreland, Pest. Biochem. Physiol. **5,** 163 (1975).
[22] J. J. S. van Rensen, van der Vet, and W. P. A. van Vliet, Photochem. Photobiol. **25,** 579 (1977).
[23] J. J. S. van Rensen, D. Wong, and Godvindjee, Z. Naturforsch. **34 c,** 413 (1978).
[24] R. Bauer and M. J. G. Wijands, Z. Naturforsch. **29 c,** 725 (1974).
[25] W. Tischer and H. Strotmann, Biochem. Biophys. Acta **460,** 143 (1977).
[26] J. Amesz, Encyclopedia of Plant Physiology, New Series, **Vol. V,** (ed. A. Trebst and M. Avron), pp. 238, Springer Verlag, Heidelberg 1977.
[27] D. I. Arnon, M. B. Allen, and F. R. Whatley, Biochem. Biophys. Acta **20,** 449 (1956).
[28] D. W. Krogmann, A. T. Jagendorf, and M. Avron, Plant Physiol. **34,** 272 (1959).
[29] J. Neumann and A. T. Jagendorf, Biochem. Biophys. Res. Commun. **16,** 562 (1964).
[30] R. Wegler, Chemie der Pflanzenschutz- und Schädlingsbekämpfungsmittel, Springer Verlag, Berlin-Heidelberg-New York, **Vol. 2,** 1970 and **Vol. 5** 1977.
[31] H. M. Cheng, M. Eto, S. Kuwatsuka, and Y. Oshima, Agr. Biol. Chem. **32,** 345 (1968).
[32] M. Avron and N. Shavit, Biochem. Biophys. Acta **109,** 317 (1965).
[33] P. H. Homann, Eur. J. Biochem. **33,** 247 (1973).
[34] A. Verloop, W. Hoogenstraten, and J. Tipker, Drug Design, **Vol. VII,** (ed. E. J. Ariens), p. 165, New York, San Francisco, London 1978.
[35] A. Trebst, Report at a workshop on "Mechanism of herbicides in photosynthesis" March 1979 at Konstanz, to appear in Z. Naturforsch.
[36] A. Trebst and H. Wietoska, W. Draber, and H. J. Knops, Z. Naturforsch. **33 c,** 919 (1978).
[37] S. J. Robinson, C. F. Yoccum, H. Ikuma, and F. Hayashi, Plant Physiol. **60,** 840 (1977).