Inhibition of Photosynthetic Electron Transport by Halogenated 4-Hydroxy-pyridines

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Herbicidal halogen substituted 4-hydroxypyridines are inhibitors of photosynthetic electron flow in isolated thylakoid membranes by interfering with the acceptor side of photosystem II. Tetrabromo-4-hydroxypyridine, the most active compound found, has a pI_{50}-value of 7.6 in the inhibition of oxygen evolution in both the reduction of an acceptor of photosystem I and an acceptor of photosystem II. The new inhibitors displace both metribuzin and ioxynil from the membrane. The 4-hydroxypyridines, like ioxynil, have unimpaired inhibitor potency in Tris-treated chloroplasts, whereas the DCMU-type family of herbicides does not. It is suggested that 4-hydroxypyridines are complementary to phenol-type inhibitors, and a common essential element is proposed.

The 4-hydroxypyridines do not inhibit photosystem I or non-cyclic electron flow through the cytochrome *b/f* complex. But they do have a second inhibition site in photosynthetic electron transport since they inhibit ferredoxin-catalyzed cyclic electron flow, indicating an antimycin-like property.

A comparison of the *in vitro* potency of the compounds with the *in vivo* potency shows no correlation. A major herbicidal mode of action of the group is related to the inhibition of carotenoid synthesis, and access to the chloroplast lamellae *in vivo* for inhibition of electron transport may be restricted.

Introduction

Many commercial herbicides interfere with photosynthetic electron flow on the acceptor side of photosystem II [1–3]. Recent advances have shown that two groups of inhibitors may be identified [4]. Firstly, a large number of herbicides represented by triazines, ureas and aminotriazones that bind to a 32 kD subunit of photosystem II [5]. Secondly, a group of inhibitors represented by phenols, like 2-bromo-4-nitrophenol and ioxynil, that bind preferentially to the 44 and 51 kD chlorophyll-peptide subunits of photosystem II [6]. Although they differ in binding characteristics and chemical reactivity, the two groups of inhibitors share common properties in blocking electron flow between Q_A and Q_B, and in displacing each other from the membrane. The first group, the urea and triazinone inhibitors, has been extensively studied in structure-activity correlations and in defining a common essential chemical element [3, 7]. There is less information on the phenol-type inhibitors [7], partly because no other group of compounds with identical inhibitory properties to those of the phenols has so far been identified.

We wish to report here that certain halogen-substituted 4-hydroxypyridines are photosystem II inhibitors analogous to the phenols. They interfere with plastoquinone reduction by photosystem II, and they displace both metribuzin and ioxynil from the membrane. Like the phenols they do not lose inhibitory potency in tris-treated thylakoid membranes, whereas the metribuzin family does. In addition to inhibiting photosystem II, the inhibitory hydroxypyridines also impair ferredoxin-catalyzed cyclic electron transport. A comparison of the in vitro potency of inhibitory 4-hydroxypyridines in isolated thylakoid membranes with their in vivo potency as herbicides, however, shows no correlation. Although several 4-hydroxypyridines are potent photosynthetic electron flow inhibitors, their herbicidal mode of

Abbreviations: DAD, diaminodurol; DBMIB, dibromothymoquinone; DCMU, dichlorophenyldimethylurea; DCPIP, dichlorophenolindophenol; DPC, diphenylcarbazide; HEPES, 4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid; MDBQ, methylenedioxydimethyl-*p*-benzoquinone; MES, 2-morpholino-ethanesulphonic acid; MV, methylviologen; PMS, phenazinemethosulfate.

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action is probably more related to the inhibition of carotenoid synthesis [8].

Materials and Methods

The halogenated 4-hydroxypyridines were synthesized by Dr. R. Bowden at ICI Mond Division, Runcorn, Cheshire, U.K. and tested for herbicidal activity at ICI Plant Protection Division.

Spinach chloroplasts were prepared by homogenizing leaves in 0.4 M NaCl, 20 mM tricine-NaOH buffer pH 8.0, and 20 mM $MgCl_2$. After centrifugation the chloroplasts were osmotically shocked in 20 mM tricine pH 8.0, 15 mM NaCl and 5 mM $MgCl_2$. Tris-treated chloroplasts were prepared essentially according to [9]. Thylakoid membranes (5 mg chlorophyll) were suspended in 10 ml of 1 M tris buffer, pH 9.0, and centrifuged down after 10 min at 0 °C. After washing in 20 mM MES buffer pH 6.5, containing 5 mM $MgCl_2$ and 15 mM NaCl they were resuspended in the same buffer.

Photosynthetic activity in the thylakoid membranes was measured either spectroscopically using ferricyanide (420 nm) and DCPIP (600 nm), or with an oxygen electrode in a methylviologen- or MDBQ-dependent Hill reaction by uncoupled (10 μg gramicidin) thylakoid membranes (equivalent to 50 μg chlorophyll) in 30 mM MES buffer pH 6.8, 10 mM $MgCl_2$ and 0.3 mM Na-azide. Cyclic photophosphorylation catalyzed by various cofactors was followed by radioactive incorporation of P^{32} into organic phosphate. 10 μM ADP and 10 μM orthophosphate (1 μC/10 μM) were added to the thylakoid membranes (70 μg chlorophyll) at pH 8.0 and 3 mM $MgCl_2$. Inhibitors were added to the probes in 10 μl methanol that was also added to the controls. PMS-cyclic phosphorylation was run for 10 min in white light, and the ferredoxin-dependent one in red light for 10 min. For redoxpoise the latter was preilluminated in the absence of DCMU for 1 min.

For the binding and displacement studies, thylakoid membranes with 100 μg chlorophyll were incubated with 0.1 μM ^{14}C-metribuzin or ^{14}C-ioxynil at pH 8.0. Further details are given in reference [6].

To determine the effects of compounds on whole-cell photosynthesis, the following procedure was adopted: Isolated mesophyll cells from asparagus plants (Asparagus sprengeri cv. Marché de Malines) were prepared as in reference [10], and adjusted to a density of 2×10^6 intact cells ml^{-1} by dilution with 10 mM HEPES buffer, pH 7.6 containing 3 mM $NaHCO_3$. 3 ml aliquots of the bulk suspension were transferred to 25 ml flasks, and test compounds added to give final concentrations of 0, 0.3, 1, 3, 10 and 30 μM. (Compounds were formulated in a mixture containing a ketone-based solvent with added wetters and emulsifiers; after appropriate dilutions, the final concentration (in the flasks) of the formulation mixture was 0.01% in all cases. Control flasks were treated with 0.01% formulation mixture only.) Samples were incubated in an illuminated water bath (21 °C, 50 μmol quanta $m-2\ s^{-1}$) for 45 min before assay. Flasks were removed for assay in the sequence: control, 4 treatments (selected at random), control, and so on. Before assaying, cell suspensions were centrifuged (10 s, $580 \times g$) and the pellet resuspended in 3 ml of fresh HEPES-bicarbonate medium plus test compound. A 2 ml aliquot was then transferred to one of a pair of calibrated oxygen electrodes (Hansatech DWl), and oxygen evolution measured over a 4 min period at 20 °C and at 200 μmol quanta $m^{-2}\ s^{-1}$ (provided by quartz-halogen fibre optics illuminators). Under these conditions, photosynthesis is rate-saturated for CO_2, and is light (electron transport) limited, which allows detection of a greater degree of inhibition by compounds affecting electron transport. A curve is constructed to establish rates of oxygen evolution against time for untreated cells, and treatment effects expressed as the percentage change from this line.

For the determination of whole plant LD_{50} values, Avena fatua (wild oat) seeds were pre-germinated to produce roots of 10 to 20 mm, and transferred to nylon mesh stretched across plastic beakers, which containing sufficient Hoaglands medium (plus micronutrients) to wet the mesh. Plants were maintained at 20 °C under a 16 h light regime (15 klux). Hydroxypyridines were added at the time of sowing as the K^+ salts dissolved in culture medium. Six dose rates and two replicates were used for each compound; (in preliminary tests the approximate LD_{50} values were determined in order to indicate the appropriate dose range). Plants were visually assessed for damage on a 0 to 9 scale 12 days after sowing.

Results

A number of halogen-substituted 4-hydroxypyridines were tested as inhibitors of photosynthetic

electron flow. The best inhibitors of this class were found to be the compounds J820 and J771. They are inhibitors of all Hill reactions, whether driven by PS I and PS II (MV), or only PS II (MDBQ). Uncoupled donor systems for PS I with (duroquinol) or without (DAD) participation of the cytochrome *b/f* complex are practically not impaired (Table I). Fig. 1 compares the inhibitory potency and the halogen-

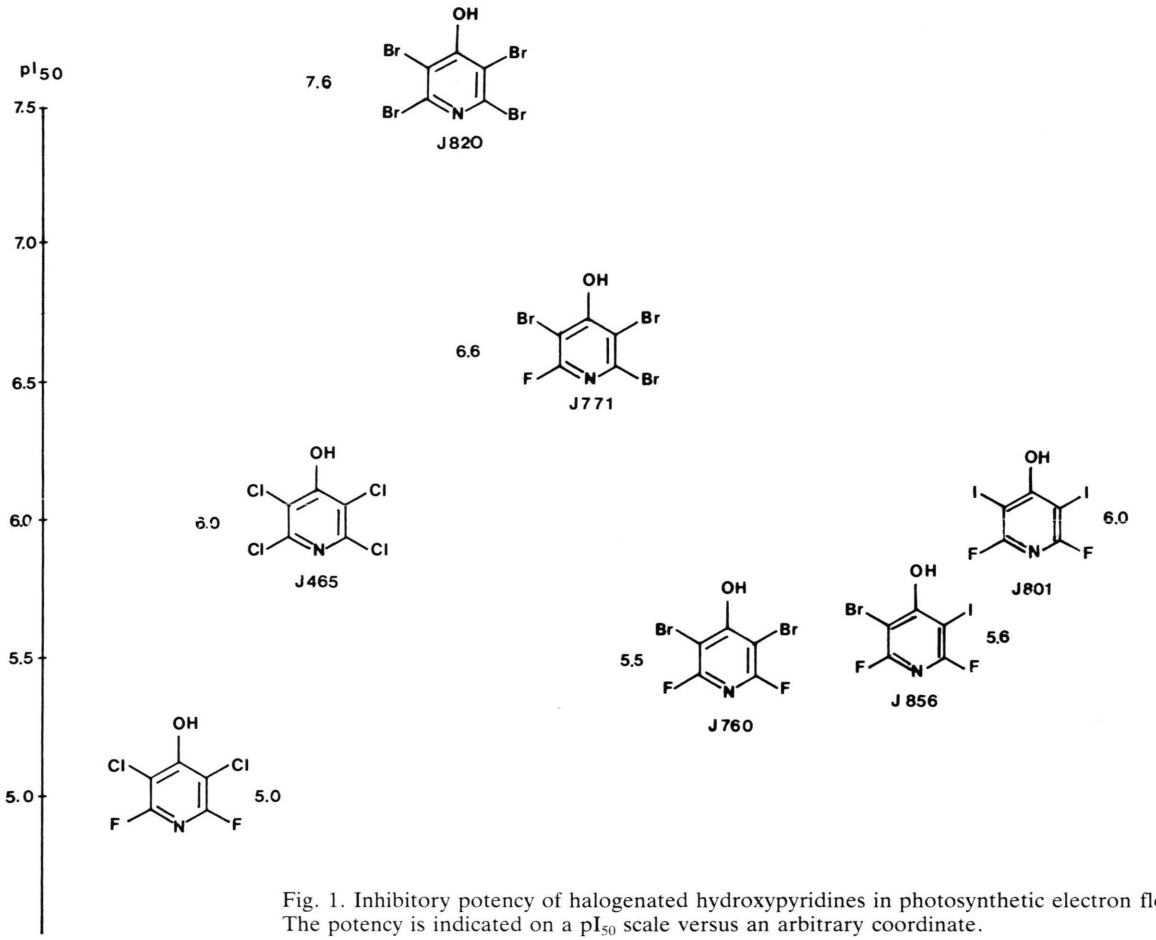


Fig. 1. Inhibitory potency of halogenated hydroxypyridines in photosynthetic electron flow. The potency is indicated on a pI_{50} scale versus an arbitrary coordinate.

Table I. Inhibition of photosynthetic reactions in thylakoid membranes by a tetrabromo-4-hydroxypyridine (J820).

Photosynthetic electron flow system measured with		Photosystem involved	% Inhibition by 1 μM inhibitor
Donor	Acceptor		
H_2O	MV	I + II	100
H_2O	Methylenedioxy-dimethyl-BQ	II	100
DAD	MV	I	0
duroquinol	MV	I + cyt *b/f* complex	0
PMS cyclic electron flow		I	2

substitution pattern of several 4-hydroxypyridines. It becomes clear that inhibitory potency falls with changes in the substitution from the most active compound, which is the tetrabromo-4-hydroxypyridine (J820), to the tetrachloro-4-hydroxypyridine (J465). The potency still diminishes if just one bromine is replaced by fluorine in the 2-position (J771), and even more so if replaced in both the 2 and 6 positions (J760). The 3,5-diiodo-2,6-difluoro substitution (J801) gives rise to a more competent inhibitor, when compared with a bromo or chloro substitution in the 3,5-position. It follows that a heavy halogen atom in the 3,5-position is particularly relevant for inhibitory potency, and it is likely that the 2 or 6 position should also be substituted by a halogen for high inhibitory potency in a photosynthetic system. None of the 4-hydroxypyridines is an uncoupler up to a concentration of 10 μM (see also Table III).

As Fig. 2 indicates, tetrabromo-4-hydroxypyridine (J820), as well as J771, displaces ^{14}C-metribuzin from the membrane. The displacement of one inhibitor by another is well known already for other herbicides like DCMU or ioxynil [2, 4]. The displacement of radioactive ioxynil by tetrabromo 4-hydroxypyridine is shown in Fig. 3. Because of the higher contribution of non-specific binding by the lipophilic phenol herbicides, the experiment differs from that in Fig. 2. The binding curve of ioxynil is changed by adding the 4-hydroxypyridine. The two experiments in Fig. 2 and 3 indicate that 4-hydroxypyridine inhibitors compete for the same binding area as metribuzin and ioxynil, *i.e.* both groups of photosystem II inhibitors share common binding regions. These properties show clearly that the hydroxypyridines have the same mode of action as the well known inhibitors of both the DCMU- and phenol-type groups: the bromo-substituted 4-hydroxypyridine are inhibitors at the acceptor side of PS II.

Tris treatment is an established method for specifically inactivating photosynthetic oxygen evolution [9]. Artificial electron donor systems for PS II, such as DPC, restore electron flow through PS II [11]. According to recent developments [12], Tris treatment is now known to remove the three peripheral peptides of 17, 23 and 34 kD that are attached to the donor side of PS II on the inner side of the thylakoid and are essential for oxygen evolution [13]. In spite of the specific effect of Tris treatment on peripheral peptides on the donor side of PS II, Tris treatment also has an effect on the acceptor side of PS II. This is indicated by the loss of inhibitor sensitivity of an electron flow system from the artificial electron donor, DPC, to the artificial electron acceptor, DCPIP. Table II summarizes the effect of a number of known herbicidal inhibitors on the acceptor side of PS II with that of the 4-hydroxypyridines. The concentration of herbicides needed to obtain 50% inhibition of electron flow in controlled thylakoids has to be increased in Tris-treated thylakoids for certain compounds, but not for others (increase in concentration is equivalent to a decrease in the pI_{50}-value). For example, a ten-fold concentration of metribuzin, or even a hundred-fold concentration of phenisopham is required in Tris-treated chloroplasts

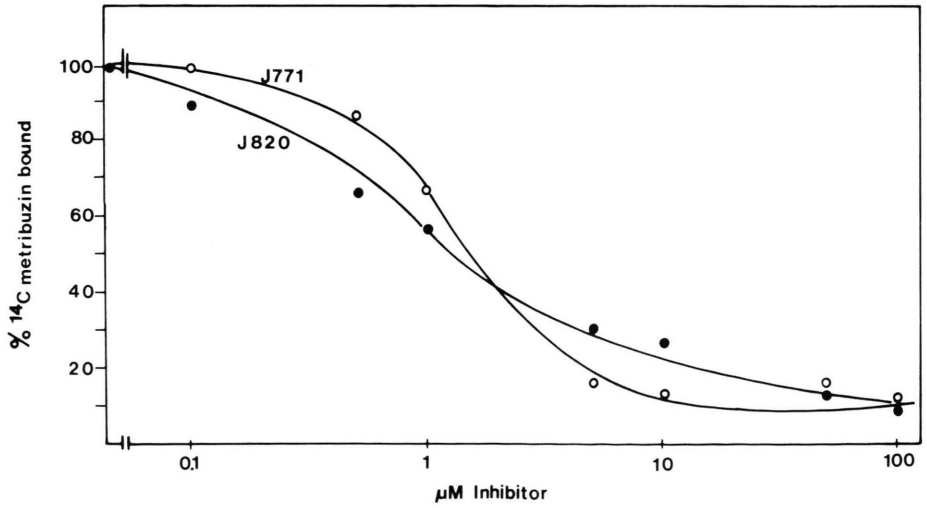


Fig. 2. Displacement of [^{14}C]metribuzin from the thylakoid membrane by halogenated hydroxypyridines.

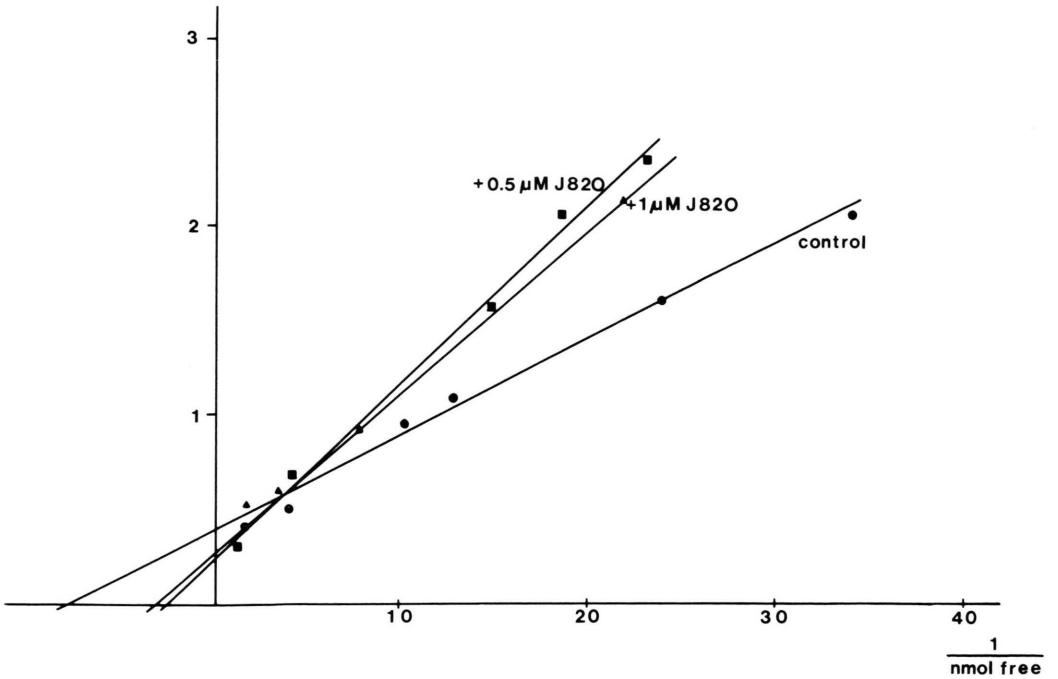


Fig. 3. Binding of [^{14}C]ioxynil to the thylakoid membrane in the presence or absence of halogenated hydroxypyridines.

to inhibit electron flow 50% as against the untreated control (Table II). This loss of inhibitory sensitivity is true for those inhibitors that are thought to interfere with the 32 kD peptide, like metribuzin, atrazine, DCMU and the biscarbamates, phenmedipham and phenisopham. In addition to lowering the pI_{50}-value, 100% inhibition of electron flow cannot be obtained in some cases at any concentration. This can be

Table II. Comparison of the inhibitory potency of two groups of PS II inhibitors in intact versus tris-treated thylakoid membranes.

		pI_{50}		pI_{70}	
	Inhibitor	Thylakoid	Tris-treated thylakoid	Thylakoid	Tris-treated thylakoid
phenol-type	tribromofluoro-hydroxypyridine (J771)	6.3	6.3	6.0	5.7
	tetrabromo-hydroxypyridine (J820)	7.6	7.8	7.0	7.3
	bromonitrothymol	7.9	7.9	7.2	7.2
	ioxynil	7.2	7.0	6.7	6.3
	iodo-nitro-isobutylphenol	7.3	7.5	7.0	6.8
atrazine-type	atrazine	6.3	5.7	6.0	5.1
	metribuzin	7.3	6.3	7.0	5.6
	DCMU	7.2	6.7	7.0	6.2
	tribunil	6.5	5.3	6.0	4.4
	phenisopham	7.3	5.3	6.0	4.5
	phenmedipham	7.7	7.1	7.2	6.8
	cyanoacrylate	7.4	6.4	7.0	6.0

pI_{50} and pI_{70} are the negative logarithms of the concentration needed for 50% or for 70% inhibition of electron flow. Photosynthetic activity measured in a DCP → DCPIP system. Cyanoacrylate = ethoxyethyl-2-cyano-3-*n*-decylaminoacrylate, kindly provided by Dr. Phillips [14].

demonstrated by choosing the pI_{70}-value, *i.e.* the concentration needed to inhibit electron flow 70% (Table II). [The pI_{70}-value is lower than the pI_{50}-value because obviously a higher concentration is needed to get 70% inhibition than to get 50%]. After Tris treatment the loss of inhibitor sensitivity towards the DCMU-type herbicides is particularly apparent in comparing these pI_{70}-values. This is due to the observation that after Tris treatment the inhibitory concentration curve for these compounds is flat, and in some cases never gets above 70%.

As against the atrazine-type inhibitors, the phenol-type inhibitors of PS II show very little change of pI_{50}- and pI_{70}-values in Tris-treated chloroplasts compared with control thylakoids. In some cases there is even an increase, *i.e.* a higher sensitivity of electron flow, after Tris treatment of the membrane. Clearly the 4-hydroxypyridine derivatives tested here belong to the phenol-group, *i.e.* they do not change inhibitory potency. Tris treatment therefore offers a way of being able to differentiate between the two groups of inhibitors. The cyanoacrylate [14] tested behaves like the triazine family of inhibitors, because its inhibitory potency changes after Tris treatment. It does so even though it does not share the common essential element of the triazine family.

The 4-hydroxypyridines are not uncouplers at doses up to 1 μM. This is shown for the most active 4-hydroxypyridines by their negligible effect on PMS-catalyzed cyclic photophosphorylation. But the 4-hydroxypyridines are inhibitors of ferredoxin-catalyzed electron flow (Fig. 4 and Table III). Their effect on cyclic electron flow is similar to that of antimycin [15]. This follows from the observation that these pyridines, like antimycin, do not block non-cyclic electron flow from the duroquinol donor system for the cytochrome *b/f* complex at the Q_z site (DBMIB site) (Tables I and III). Therefore it can be concluded that the 4-hydroxypyridines are inhibitors at the Q_c site (antimycin site) of this complex, at the second quinone binding site on the complex by which reduced ferredoxin donates electrons into the system in cyclic electron flow.

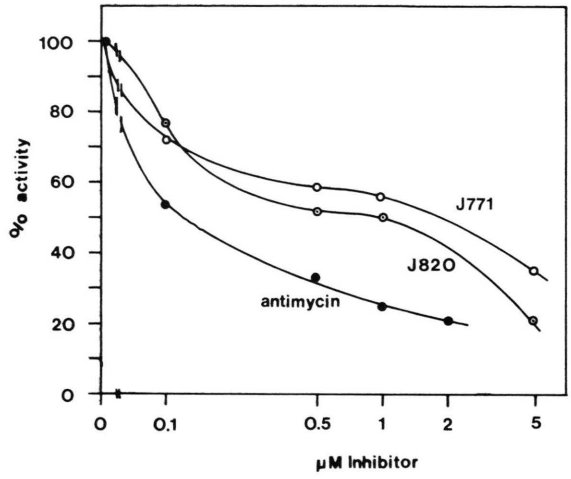


Fig. 4. Inhibition of ferredoxin catalyzed cyclic electron flow by antimycin and halogenated hydroxypyridines (measured as ATP formation as in Table III).

Table III. Inhibition of cyclic electron flow by a halogenated hydroxypyridine.

Cofactor of the cyclic electron flow:		PMS (0.1 mM) ATP [μmol]	% Inhibition	Duroquinol (0.5 mM) ATP [μmol]	% Inhibition	Ferredoxin (0.25 mM) ATP [μmol]	% Inhibition
control		4.0		3.3		0.71	
inhibitor	concentration (μM)						
DBMIB	1	3.4	15	0.1	97	0.01	90
antimycin	0.1					0.38	46
	0.5					0.24	66
	1	4.0	–	3.25	–	0.18	74
J771	0.1	3.9	–			0.5	28
	0.5	4.1	–			0.42	41
	1	4.0	–	3.2	–	0.36	41
	5	3.9	–			0.15	78

Comparison of the sensitivity of three cyclic electron flow systems towards antimycin and tribromofluorohydroxypyridine (J771) in the presence of 2 μM DCMU. Rates are given as μmol ATP formed in 10 min light by thylakoid membranes with 70 μM chlorophyll.

A broader aspect of 4-hydroxypyridine-inhibition of electron transport has also been considered because of the need to understand how this relates to their primary herbicidal mode of action. The question has been raised [8] as to whether the 4-hydroxypyridines ever get into the chloroplasts in whole plants to exert this type of inhibition. To try and answer the question, isolated single cells of asparagus (actively photosynthesizing) have been treated with varying doses of the two 4-hydroxypyridines (J820 and J771) that cause the most inhibition of electron transport in isolated thylakoids. These have been compared with ioxynil, a potent inhibitor in the same phenol-type group. The results are shown in Table IV, and confirm that all three compounds inhibit light-dependent oxygen evolution. Ioxynil is clearly much more potent than either of the 4-hydroxypyridines, and gives 50% inhibition at about 0.7 μM. The tetrabromopyridine, J820, is about 3-fold more active than J771. Approximately 10 μM J820 is needed to achieve 50% inhibition of asparagus cells, whereas about 30 μM J771 gives 50% inhibition. The marked difference in the effect of these compounds on photosynthetic activity in comparison with ioxynil strongly suggests that the ability of the hydroxypyridines to reach the chloroplast lamellae is restricted. This is further born out when comparing different 4-hydroxypyridines for their herbicidal effects against *Avena fatua* (wild oat). Table V clearly demonstrates that J820 (the most potent at inhibiting electron transport) is the weakest herbicide, whilst haloxydine (the least potent at inhibiting electron transport) is the most potent herbicide. The main herbicidal symptom is chlorosis which is of the type characteristic of inhibition of carotenoid syntheses [8]. With J820 (but not J771), the symptom of photo-

Table IV. Effects of 4-OH-pyridines and ioxynil on light-dependent O_2 evolution from intact asparagus mesophyll cells. Figures represent percentage change from controls containing formulation.

Rate [μM]	Compound J820	J771	Ioxynil
0.3	+ 1	NT	− 12
1.0	− 1	+ 2	− 74
3.0	− 21	− 7	− 109*
10.0	− 52	− 22	− 115*
30.0	NT	− 48	NT

NT, Not tested.
* Net oxygen consumption: respiration exceeds photosynthesis.

Compound	LD_{50} [μM
J820	5.60
J771	0.169
J465	0.237
J801	0.337
J760	0.062
J856	0.140
Haloxydine	0.055

Table V. Comparative herbicidal effects of several halogenated 4-hydroxy-pyridines on *Avena fatua* (wild oat). Chemical structures are shown in Fig. 1.

destruction of pre-existing chlorophyll is also found, and this is typical of PS II inhibitor herbicides, indicating that two different modes of action are being expressed simultaneously with this compound. Even so, the undoubted ability of these two 4-hydroxypyridines to inhibit electron transport *in vitro* is not being fully expressed *in vivo*.

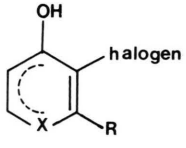
Discussion

Two groups of inhibitors on the acceptor side of PS II may be distinguished that functionally interfere between the primary quinone Q_A and the secondary quinone Q_B [see 4, 7]. Both groups bind to a common binding area and displace each other from the membrane. From these two groups, one that includes a large number of herbicides like diuron and triazine with a common essential element, an sp^2 carbon [1, 3, 7], binds to a 32 kD subunit of PS II [5]. The inhibitory potency of this group of herbicides is governed predominantly by electronic and lipophilicity parameters, and shows a definite π charge density at the atoms essential for binding [16]. The second group are phenol-type inhibitors. They have different structure-activity correlations from the first [7] (they follow steric parameters rather than electronic parameters) and they have a different electron density distribution at the essential atom [16]. Their binding to the PS II components occurs predominantly on the larger 44 and 51 kD subunits of PS II [6]. Their binding to the membrane is shown to be more stable even after trypsin treatment of the membrane, and is still present in PS II preparations in which the 32 kD peptide is dislodged from the reaction centre peptides; therefore the DCMU sensitivity, but not the phenol sensitivity, is lost [6, 17, 18].

This report describes another group of compounds that belong to the group of PS II inhibitors: halogen-substituted 4-hydroxypyridines. Such compounds have been in the literature as herbicides for some time [1, 3]. One of us has already reported on the high inhibitory potency of the Hill reaction by the

tribromofluoropyridine (J771) [8]. Pyriclor, *i.e.* 2,3,5-trichloro-4-hydroxypyridine, had also been found to interfere with photosynthesis [19]. The studies here indicate that 4-hydroxypyridines inhibit PS II, but belong to a distinctly different group of PS II inhibitors than the classical herbicides of the DCMU-type. Instead, 4-hydroxypyridines complement the phenol-type inhibitors of PS II. This points to the emergence of another, probably large, group of potential inhibitors of photosynthesis that follow different structure-activity correlations from the well known PS II herbicides, although they interfere with PS II close to the site of the DCMU-type herbicides. This new group of compounds also interferes with the acceptor side of PS II, since they displace both radioactive ioxynil as well as metribuzin from the membrane; thus they follow the described effect of phenol inhibitors [6, 18]. Their specific binding niche in the common binding area is therefore close to or identical to that of the phenol rather than the metribuzin binding site. This conclusion is reached particularly from the inhibitory efficiency of 4-hydroxypyridines in tris-treated thylakoid membranes. In these membranes the inhibitory potency of the diuron and metribuzin family is diminished, whereas that of the phenols and the 4-hydroxypyridines is not. This system therefore provides an easy check to discriminate between the two groups.

It is already tempting to speculate on a common essential element in the two classes of compounds: a hydroxy group (or tautomeric carbonyl group) attached to a vinyl group substituted by a halogen or nitro group. This carbon will not carry the positive π charge, found in the DCMU-type inhibitors and thought to be essential there [16]. The hydroxy and vinyl group are part of a cyclic system, which may be five or six membered and may or may not contain a heteroatom.



Among the compounds that also follow these rules are, for example, quinones and quinolones. Indeed, hydroxyquinoline-N-oxide [20] and substituted naphthoquinones [21] have already been shown to be PS II inhibitors.

The effect on the properties of the acceptor side of the water splitting system when peripheral peptides are removed by tris treatment of the membrane (as shown by changes in the pI_{50}-values of inhibitors) is rather interesting. It is in accordance with the recent prediction of membrane folding from the amino acid sequence [22] that the 32 kD peptide is a membrane-spanning peptide with hydrophilic amino acids protruding from either side of the membrane. Therefore the 32 kD subunit can sense a change in peptide environment brought about on either side of the membrane, and can relay it through the membrane to its amino acids outside, which are involved in herbicide binding. An effect of an internal amino acid sequence of the 32 kD peptide on the functional integrity of the plastoquinone and herbicide binding sites oriented towards the matrix side is also indicated by the serine 264 mutation in the hydrophilic sequence located on the inside of the membrane in atrazine-tolerant plants and algae [23, 24]. Another explanation follows the recent suspicion that the herbicide binding peptide is part of the photosystem II reaction center and therefore has the peripheral peptides directly attached that are removed by tris treatment.

The 4-hydroxypyridine herbicides are not uncouplers as the phenols are. Therefore it is possible to investigate their effects on ferredoxin-catalyzed cyclic photophosphorylation. This system has a particular electron flow pathway through the cytochrome *b/f* complex that is not necessarily also participating in steady state non-cyclic electron flow. It is this part of the cytochrome *b/f* complex where antimycin sensitivity resides [25]. The ferredoxin-catalyzed cyclic system is the only photosynthetic system that is antimycin sensitive [15]. It is shown here that the 4-hydroxypyridines, J820 and J771, are also good inhibitors of this cyclic system. As 4-hydroxypyridines do not inhibit the duroquinol donor system (that also feeds electrons into the Q_Z site of the cytochrome *b/f* complex), it is concluded that the pyridines are not inhibitors like DBMIB, but are analogous to antimycin as inhibitors of the Q_C site at the cytochrome *b/f* complex. A more detailed discussion of this system with more experimental documentation (by A. Hartung and H. Wietoska) is to be reported elsewhere (Physiol. vegetale, in press). One of us has already pursued the idea that the effect of herbicides on the cyclic electron flow is of particular relevance for herbicidal action [26].

A comparison of absolute potency of the two 4-hydroxypyridines, J820 and J771, with ioxynil (a member of the same group) on photosynthesis in iso-

lated whole cells from asparagus provides a rather different picture from that obtained using isolated thylakoid membranes. Furthermore, the herbicidal potency against a susceptible plant (wild oat) bears no relationship to the *in vitro* potency against the electron transport system. This may be due in some degree to differences in cellular penetration in wild oat, but the 4-hydroxypyridines are herbicidal for a number of different reasons, and it is likely that their different sites of action are located within different compartments. One of the major actions is the inhibition of carotenoid synthesis [8] (as also revealed by the symptom of chlorosis), but the ability of these compounds to reach the chloroplast lamellae seems to be limited, and it is probable that the carotenoid inhibition site is in the cytoplasm [8]. Only when the 4-hydroxypyridine is also such a highly potent inhibitor of electron transport as J820 will symptoms associated with that type of inhibition (photodestruction of pre-existing chlorophyll) appear as well. It should be noted that not all 4-hydroxy halopyridines are electron transport inhibitors, for example, the tetrafluoro 4-hydroxypyridine does not inhibit electron transport, but causes stunting in whole plants for reasons that are not yet known [8].

In its effects on chloroplast lamellae, J820 possesses the potential properties considered to be essential for a PS II-type herbicide to induce photodestruction [26]: potent inhibition of non-cyclic electron flow close to PS II, and at the same time the ability to block cyclic electron flow at a second binding site. Diuron inhibits cyclic flow (and induces photodestruction), but only when non-cyclic electron flow is blocked (at its primary binding site) almost completely. This is probably caused solely by the redox poising being disrupted, rather than by additional action at a second binding site. However, as our evidence shows, J820 has the additional ability to block cyclic electron flow separately, at a binding site similar to that of antimycin. If J820 were able to get into the chloroplast easily, the simultaneous expression of these two effects in whole plants should provide a considerable degree of synergy in terms of induced photodestruction, and hence greater herbicidal efficacy than that which is actually observed.

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