The Effects of Photosystem I Electron Acceptors on Leaf Discs

P. Camilleri*, J. R. Bowyer**, and P. H. McNeil*

* Shell Research Limited, Sittingbourne Research Centre,
Sittingbourne, Kent ME9 8AG, U.K.
** Department of Biochemistry, Royal Holloway and Bedford New College,
Egham Hill, Egham, Surrey TW20 OEX, U.K.

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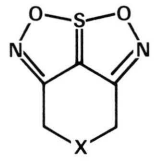
A leaf-disc chamber has been used to measure changes in photosynthesis by soybean leaf discs which have been supplied with photosystem I (PS I) electron acceptors *via* the transpiration stream. The uncharged heteropentalenes induce an inhibitory effect on CO_2 fixation which is dependent on the *n*-octanol-water partition coefficient of these molecules. Paraquat, which carries a di-positive charge, was ineffective under the same conditions and at a concentration of 10^{-4} M. Cationic species such as K^+, Ca^{2+} and Mg^{2+} greatly enhanced the effect of paraquat on CO_2 fixation while the presence of these ions did not affect inhibition by the heteropentalenes. Such differences between uncharged and charged PS I electron acceptors have been explained in terms of electrostatic interactions encountered by paraquat on its way to the site of action within the chloroplast, *via* the transpiration stream.

Introduction

Over the last two decades several laboratories have devoted considerable effort in the search for compounds that can exert herbicidal effects by accepting electrons from chloroplast photosystem I (PS I), in direct competition with the natural substrate ferredoxin. Bipyridinium cations, in particular paraquat (PQ^{2+}, also called methyl viologen) and diquat, which have redox potentials between −300 and −550 mV (*vs.* N.H.E.) accept an electron from PS I giving rise to a bipyridyl cation radical [1]. The latter species reacts rapidly with oxygen [2] (k = 7.7×10^8 $dm^3\ mol^{-1}\ s^{-1}$ in the case of paraquat cation radical, PQ^+) to give superoxide ($O_2^{\cdot-}$) regenerating once more the bipyridinium cation. The superoxide anion radical is not considered to be toxic to the chloroplast system. However, this species is thought [3] to convert to other toxic species, such as the hydroxyl radical ($HO^{\cdot}$) and hydrogen peroxide, by Fenton type reactions. Interactions of these reactive oxygen species with allylic hydrogens of unsaturated membrane lipids both in the chloroplast thylakoids and in the chloroplast envelope lead first to the formation of unstable hydroperoxides and eventually to the destruction of the plant.

In the course of a search for compounds that interfere with the photosynthetic electron transport system in the same way as the bipyridinium herbicides, we discovered a number of dioxathiadiaza-heteropentalenes. The chemical structures of seven of these compounds are shown below.



HEP I X = CH_2
HEP II X = S
HEP III X = SO
HEP IV X = SO_2
HEP V X = $C(CH_3)_2$
HEP VI X = $CH(CH_3)$
HEP VII X = $CH(C_6H_5)$

As we reported previously [4, 5] these molecules can be electrochemically reduced at a potential comparable with the first reduction of the bipyridinium herbicides (see Table I) and act as PS I electron acceptors at concentrations of the order of 10^{-6} M. Using electron spin resonance spectroscopy, we also showed [6] that $O_2^{\cdot-}$ was formed when pea thylakoids were illuminated in the presence of one of these heteropentalenes (HEP II). An important difference between the bipyridinium salts and the heteropentalenes is the electrical neutrality of the latter. In fact, using the oxygen electrode to monitor oxygen uptake, we had found [6] that magnesium ions (Mg^{2+}) decreased the rate of oxygen uptake with paraquat, but not with HEP II, probably reflecting differences in the interaction of these molecules with the PS I electron donation site.

Reprint requests to P. Camilleri.

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To evaluate further the possible role played by the hydrophobicity of the heteropentalenes in their interference of photosynthetic electron transport, we have measured the *n*-octanol-water partition coefficient of HEP I to HEP VII and have related this property to the inhibitory effect of these molecules on photosynthesis by illuminated leaf discs.

Materials and Methods

A chamber which permits chemicals to be supplied to leaf discs *via* the transpiration stream was fitted into an open infra-red gas analysis system as shown in Fig. 1. A Watson-Marlow HR Flow Inducer was used to pump either water or the required chemical around the cut surface of the leaf disc and leaf temperature was constantly monitored by a thermocouple resting on the underface of the leaf. The upper surface of the leaf was illuminated using a Volpi HL250 fibre optic system. Light from this system was passed through a series of lenses, heat absorbing glass and heat reflecting mirrors to allow a uniform beam of relatively "cold" light to illuminate the surface of the disc. The intensity of the light arriving at the leaf surface was measured as 1500 $\mu Em^{-2}\,s^{-1}$.

Leaf discs (diameter 5.4 cm) were cut from soybean leaves *(Glycine max.)* and were floated on water under light for at least 1 h before being placed in the chamber in order to enable the stomata to open fully. HEP I to HEP VII and paraquat were dissolved in a suitable solvent at 10^{-2} M and then diluted with water to give 10 ml of a 10^{-4} M solution. This solution was pumped round the leaf chamber and constantly recirculated during an experiment.

n-Octanol-water partition coefficients *(P)* of the heteropentalenes were determined by the "shake-flask" method, using deionized water and "Analar" *n*-octanol; the solute concentration in the water and octanol phases were analyzed by absorption spectroscopy. The values of log *P* obtained for HEP I to HEP VII are given in Table I and the range between the lowest and the highest value for *P* is about a thousand.

Results and Discussion

The effect of some heteropentalenes on photosynthetic carbon dioxide uptake by soybean leaf discs is shown in Fig. 2. It is apparent that the heteropentalenes with the lowest octanol-water partition coefficient (highest water solubility), for example HEP IV and HEP III, acted the most rapidly. Molecules with a partition coefficient greater than about 1.4 inhibited photosynthesis very slowly under the same conditions (see Fig. 3). These results are at variance with findings in the Primary Screen (Table II) in which compounds are supplied to the leaf surface. In this case, HEP II with a partition coefficient of 1.2 is the most phytotoxic compound. An important difference between conditions employing leaf discs and the Primary Screen (foliar application) is that, in the former case, the herbicide does not have

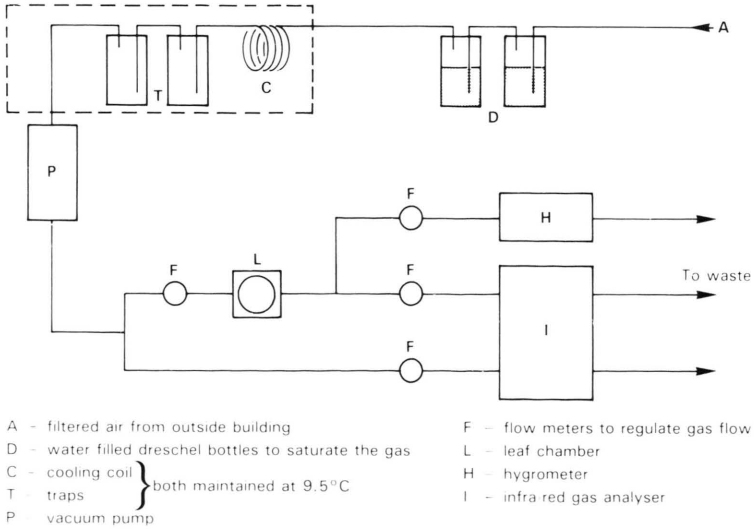

Fig. 1. Diagramatic representation of gas supply system.

Table I. A summary of the physico-chemical properties of heteropentalenes I to VII, paraquat and diquat.

Compound	$-E^1_7$ [mV]	$-E_{p/2}$ (MeOH/water 3:1 by volume) (mV *vs.* Ag−AgCl)	pKa ($AH^\circ/A^{\circ-}$)**	*k* ($O_2 + A^{\circ-}$) [$dm^3\ mol^{-1}\ s^{-1}$]	log *P* (*n*-octanol/ water)
HEP I	416	693	5.9	3.5×10^8	1.1
HEP II	375	643	4.5	4.5×10^8	1.2
HEP III	277	573	0	1.2×10^8	−0.30
HEP IV	227	523		0.57×10^8	0.35
HEP V	–	713	–	–	1.9
HEP VI	–	683	–	–	1.5
HEP VII	–	673	–	–	2.8
Diquat	350	545*	–	4.7×10^8	<0
Paraquat	455	650*	2.9	7.7×10^8	<0

* In water at neutral pH.

** Anion radial ($A^{\circ-}$) derived from heteropentalenes.

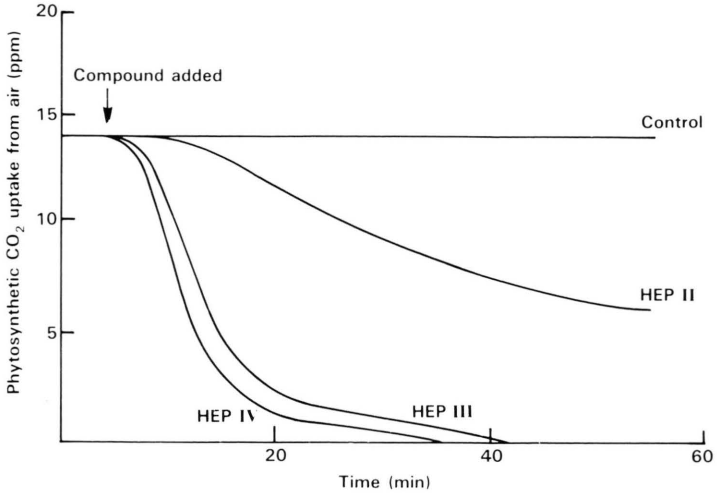

Fig. 2. Effect of some heteropentalenes on photosynthesis by soybean leaf discs.

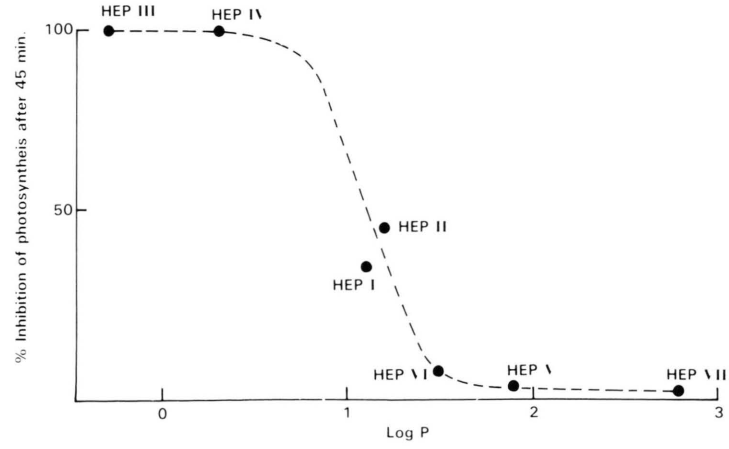

Fig. 3. Relationship between octanol-water partition coefficient (P) of heteropentalenes and their inhibitory effect on photosynthesis by soybean leaf discs.

Table II. Herbicide primary screen results.

Test compound	Dose [kg/ha]	Foliar spray*								Total score
		MA	RI	BG	OA	LI	MU	SB	SO	
HEP I	5	5	5	8	6	9	9	9	8	59
	1	3	3	6	3	6	7	7	3	41
HEP II	5	8	9	9	8	9	9	9	9	70
	1	5	8	9	6	8	9	9	8	62
HEP III	5	8	9	9	8	9	9	9	9	70
	1	6	7	9	5	7	9	9	8	60
HEP IV	5	7	8	9	8	9	9	9	9	68
	1	4	6	8	6	8	9	5	7	53
HEP V	5	3	5	9	6	9	9	9	7	57
	1	2	1	8	1	4	6	6	5	33
HEP VI	5	5	4	9	6	8	9	9	8	58
	1	4	3	8	4	7	8	8	5	47
HEP VII	5	0	0	3	0	3	3	2	2	13
	1	0	0	1	0	0	2	1	0	4
Paraquat	5	9	9	9	9	9	9	9	9	72
	1	8	9	9	9	9	9	9	9	71

* Plant species mentioned: MA, maize *(Zea mays);* RI, rice *(Oryza sativa);* BG, barnyard grass *(Echinochloa crus-galli);* OA, oat *(Avena sativa);* LI, linseed *(Linum usitatissimum);* MU, mustard *(Sinapsis alba);* SB, sugar beet *(Beta vulgaris);* SO, soybean *(Glycine max.).*

to penetrate the epicuticular wax on the leaf surface. The leaf-disc experiments demonstrate that when this barrier is bypassed, movement of the more water soluble compounds HEP III and HEP IV to the plant cells is very rapid. It appears that HEP II strikes the best balance between penetration through the epicuticular waxes of the leaf surface and freedom of movement within the plant.

When paraquat (10^{-4} M) was supplied to the illuminated leaf disc it had no effect on photosynthesis even after 60 min (Fig. 4a). This perhaps surprising result is however consistent with the known slow rate of translocation of bipyridinium salts from their site of application in the light. When Mg^{2+} was included in the paraquat solution, inhibition of photosynthesis was observed after 10 min, and the degree of inhibi-

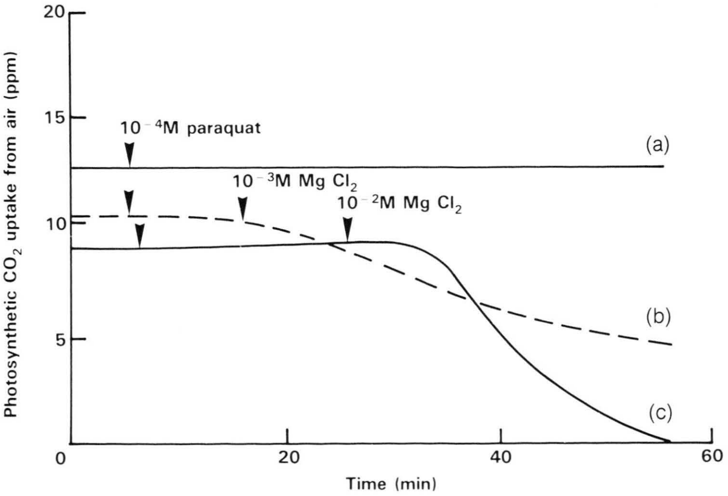

Fig. 4. The effect of $MgCl_2$ on paraquat activity in the leaf chamber. (↓) indicates the time when either paraquat or $MgCl_2$ was added.

tion increased as the concentration of Mg^{2+} was increased (Fig. 4b, c). Ca^{2+} had a similar effect (not shown), and K^+ was much less effective at the same concentration (not shown). Divalent cations had no effect on the efficacy of the heteropentalenes.

Since the heteropentalenes are neutral in the oxidized state, whereas paraquat carries two positive charges, we interpret the effects of divalent cations as indicating that paraquat binds electrostatically to negatively charged surfaces within the plant, presumably outside the chloroplast but possibly within the vascular system. Divalent cations render paraquat more mobile by competitively binding to these negatively charged surfaces. The electrostatic interactions of paraquat with negative surface charges can therefore affect both its movement within the plant, as well as its interaction with PS I [6], as discussed in the Introduction.

Presumably the high toxicity of paraquat when applied by foliar application is a reflection of the smaller distance it needs to traverse to reach its site of action in comparison to the leaf-disc application. Differences in cation concentration in the vascular system between illuminated and non-illuminated leaves may explain why paraquat is more mobile in plants maintained in darkness [7]. These results may also give clues to the basis of paraquat-resistance in *Conyza bonariensis*. Although paraquat binds equally to extracted cell walls from resistant and susceptible biotypes [8], this does not preclude differential electrostatic binding to some other component in the plant. Such a model could be tested by applying an uncharged heteropentalene to leaf discs from susceptible and resistant plants.

Conclusions

When heteropentalenes are supplied to leaf discs *via* the transpiration stream they inhibit photosynthetic CO_2 fixation by channeling electrons from PS I to oxygen. Activity increases with decreasing *n*-octanol-water partition coefficient (*i.e.* with increasing water solubility). When applied to the foliar surface, however, maximal phytotoxic activity is observed with a heteropentalene with an intermediate value of partition coefficient, presumably reflecting a balance between penetration of the epicuticular wax, and movement within the vascular system. Paraquat is not effective when supplied *via* the transpiration stream, unless divalent cations are also supplied. The absence of any effect of divalent cations on neutral heteropentalenes suggests that divalent cations render the paraquat dication more mobile by displacing it from negatively charged surfaces.

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