OPTICALLY ACTIVE PHOTOSYNTHETIC INHIBITOR HERBICIDES

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Summary. Optically active cyanoacrylate derivatives inhibit photosynthetic electron transport in pea and brassica chloroplasts. The S-isomer is more active than the R-isomer by one to two orders of magnitude, suggesting a stereo-specific interaction between the inhibitor and the receptor site. Both S and R-isomers are more effective inhibitors of electron transport in chloroplasts isolated from atrazine susceptible than from atrazine resistant brassica biotypes, and there is a general correlation between their electron transport inhibitory activity and their herbicidal effectiveness.

INTRODUCTION

Many photosynthetic herbicides including diuron, atrazine, propanil, bromacil and metribuzin are known to inhibit photosynthetic electron transport (PET) in isolated chloroplast systems (1). They are believed to act by displacing the plastoquinone electron acceptor (Q_b) from its binding niche on the D_1(32KD) peptide associated with the photosystem II (PS_{II}) reaction centre of the thylakoid membrane. Such an inhibitor-peptide interaction would be expected to be highly stereo-specific and thus discriminate between optically active isomers. Suprisingly, however, although PS_{II} PET inhibitors have been known since the early 1950's, few optically active derivatives have been studied.

Moreland and Boots, (2) using a diuron analogue, were the first to differentiate the PET inhibitory activities of R and S-optical isomers. They reported the S-isomer to be about ten times as potent a PET inhibitor as the R-isomer in isolated spinach chloroplasts although neither isomer was herbicidally active. Later, Gardner and Sanborn (3) reported a similar level of discrimination between optical isomers of a series of triazine derivatives and showed a correlation between PET inhibitory activity and herbicidal activity.

METHODS

Ethoxyethyl-2-cyano-3-ethyl-3-α-methylbenzylamino acrylate (I) and its S and R-isomers, arising from the asymmetric (chiral) centre on the benzylic carbon atom, have been described elsewhere (4).

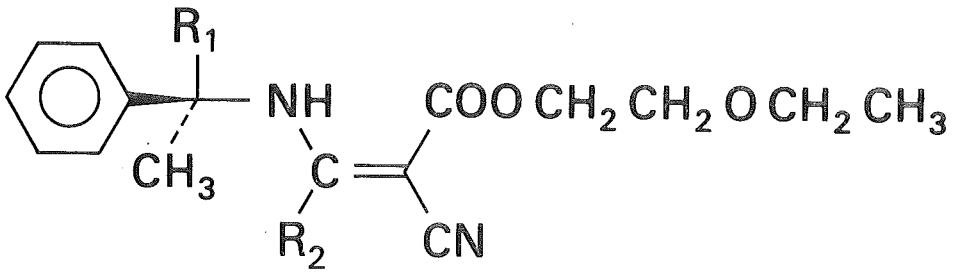

Figure 1. Structural formula of I

The activities of these compounds as inhibitors of photosynthetic electron transport in the Hill reaction were determined with chloroplasts isolated from peas, *Pisum sativum*, and atrazine susceptible (wild type) and atrazine resistant (mutant) rape, *Brassica napus*, species. Both brassica species, field isolates from Canada, were supplied by Dr J.T. Kirk of the CSIRO, Division of Plant Industry.

Hill reaction studies were carried out using 2,3,6-trichlorophenol-indophenol as the electron acceptor following the technique of Brown *et al.* (5). Results were expressed in terms of pI_{50} values, i.e., as the negative logarithm of the molar concentration of inhibitor giving 50% of the uninhibited reaction rate.

Herbicidal activities were determined using mustard, *Sinapis alba*, and the wild type and mutant brassica species described above. Glasshouse grown fourteen day old seeedlings were sprayed to run off with 50% aqueous acetone solutions of the compounds at rates equivalent to 0.125, 0.25, 0.5, 1, 2, 4, 8 kg/ha. Results were expressed in terms of ED_{90} values, i.e., the lowest application rate giving $\geq$90% kill.

RESULTS AND DISCUSSION

Cyanoacrylates, a recently discovered group of photosynthetic herbicides (6), have proved convenient for studying optically active PET inhibitor isomers (4). Table 1 records the PET inhibitory activities, expressed as pI_{50} values, for the racemate (I) and its R and S-optical isomers in chloroplasts isolated from peas and from wild type and mutant brassica species.

Table 1. Herbicidal and PET inhibitory activities of I and its S and R-isomers.

Compound	Pea	pI_{50} Brassica (wild type)	Brassica (mutant)	Mustard	ED_{90} (kg/ha) Brassica (wildtype)	Brassica (mutant)
Racemate (I)	6.9	7.1	5.1	0.5	0.5	>8
S isomer (I)	7.1	7.75	5.85	0.25	0.25	>8
R isomer (I)	4.9	5.4	4.05	>8	>8	>8

The data in Table 1 indicate:

1. There is a large difference between the PET inhibitory activities of the S and R-isomers with the level of discrimination varying from one to two hundred fold in peas and wild type brassicas to around ten fold in the mutant brassicas. This would be in accord with a stereo-specific interaction between the inhibitor molecule and a peptide.

2. In all cases the S-isomer is the more active PET inhibitor indicating that the molecular nature of the receptor site is broadly similar in the species studied. The activity of the racemate is consistent with an equi-molar mixture of S and R-isomers.

3. The racemate and its S and R-isomers are ten to a hundred fold more

effective PET inhibitors in wild type than in mutant brassica chloroplasts. In this respect they behave more like atrazine which shows a 500-1000 fold discrimination in favour of inhibiting the wild type than like diuron which is virtually unable to distinguish between the biotypes. It is known that the amino acid sequence in the mutant biotype differs from that in the wild type in only one amino acid residue; serine (264) in the wild type being replaced by glycine in the mutant (7). Since both optical isomers discriminate between the biotypes in a similar way it is unlikely that the chiral centres of these inhibitors interact closely with the altered amino acid region of the peptide.

Compound I has been found to be herbicidally active against a range of plant species when applied post-emergence at rates of 0.2-1 kg/ha. These species include swamp chinese lantern, *Abutilon theophrasti*, redroot amaranthus, *Amaranthus retroflexus*, Indian mustard, *Brassica juncea*, fat hen, *Chenopodium album*, cleavers, *Galium aparine*, morning glory, *Ipomea* spp., wireweed, *Polygonum aviculare*, common groundsel, *Senecio vulgaris*, green pigeon grass *Setaria viridis*, and Noogoora burr, *Xanthium strumarium*

The ED_{90} data in Table 1 indicate that compound I in its racemic form is also phytotoxic towards *Sinapis alba* and wild type *Brassica napus*. This largely reflects the phytotoxicity towards these species of the S-isomer which is much greater than that of the R-isomer. Such isomer discrimination in respect of herbicidal activity is in accord with their relative PET inhibitory activities. Likewise the greater sensitivity of the wild type as compared with the mutant brassica species to both the S-isomer and the racemate is consistent with the pI_{50} values observed using chloroplasts isolated from these species. A correlation between the herbicidal and PET inhibitory activities of optical isomers is to be expected since the physico-chemical properties of the isomers and hence their transport and permeability behaviour within the plant is likely to be very similar (8).

Cyanoacrylates differ from most classical PET inhibitors in that chiral centres can be introduced into various sites in the molecule. Pairs of optical isomers of known stereochemical configuration can then be generated. Such optically active PET inhibitors are useful for probing the three dimensional molecular architecture of the PS_{II} reaction centre and the herbicide binding domain and provide a basis for the rational design of future photosynthetic PS_{II} herbicides.

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