The Role of Chirality in the Activity of Photosystem II Herbicides

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Although significant differences in activity between optical isomers have been recognized in many types of pesticides, the role of stereoselectivity has not been fully characterized for one of the most important classes of commercial herbicides, those that inhibit photosynthetic electron transport. This report describes experiments in which optically active α-methylbenzylamine or *sec*-butylamine was used as starting material for the synthesis of optically active triazine and urea herbicides. The biological activities of the compounds were determined in two *in vitro* chloroplast assays – competition for specifically bound [^{14}C]atrazine and inhibition of photosystem II-mediated dye reduction – as well as in whole plant phytotoxicity. In both *in vitro* assays the (−)-isomer of the N-α-methylbenzyl triazine was about 15-fold more active than the (+)-isomer, and the racemate fell in between and was of about the same potency as atrazine. The same relative activities were also seen for *in vivo* phytotoxicity. The α-methylbenzyl urea derivatives were much less herbicidally active, but the *in vitro* assays were able to discriminate between the optical isomers. In both assays, the (−)-isomer of the urea was much more active than the (+)-isomer, with the racemate intermediate. Steric factors play a critical role in the degree of this chiral discrimination, since in both the corresponding triazines and ureas, the optically active molecules synthesized from the enantiomers of 2-butylamine showed only slight differences in activity. Saturation of the phenyl ring of the α-methylbenzyl triazines resulted in molecules which still showed substantial differences in activity related to chirality, further supporting the importance of steric factors, rather than electronic, in this chiral discrimination.

Introduction

Chiral recognition plays an important role in the high activity and selectivity of modern pesticides. Great differences in activity between optical isomers have been recognized in many types of insecticides, including the pyrethroid, fenvalerate [1]. Stereoselectivity is less well documented for herbicides, although this property is extremely important for the activity of the wild oat herbicide, flamprop-isopropyl [2]. Among the most important classes of commercial herbicides are those that act by inhibiting photosynthetic electron transport at the second electron carrier on the reducing side of photosystem II. These compounds include the chemical classes of ureas, amides, triazines, triazinones, pyridazinones, carbamates, and nitrophenols. Although structure-activity relationships for photosystem II (PS II) inhibitors have been reviewed on many occasions (*e.g.*, ref. [3]), prior to the inception of this work, there was only one report [4] on the role (or lack thereof) of stereoselectivity in the inhibition of photosynthetic electron transport. This question had probably not been addressed in detail because most of the commercially-important PS II inhibitors, including atrazine, cyanazine, and diuron, do not contain an assymetric carbon atom.

This report probes the role of stereoselectivity in inhibition of photosynthetic electron transport. Optically active triazine and urea herbicides were synthesized from optically active starting materials, and their affinities for the site of action on chloroplast thylakoid membranes were measured directly in two *in vitro* assays: competition for specifically bound [^{14}C]atrazine [5] and inhibition of photosystem II-mediated dye reduction.

Materials and Methods

Chloroplast membranes were prepared from seven- to eight-day-old Alaska peas (*Pisum sativum* L., cv. Alaska) as previously described [6]. Atrazine binding experiments were carried out [7] using either 0.1 or 0.2 μM [^{14}C]atrazine and higher concentrations of nonradioactive compound (or 1% MeOH) as noted. "Specific" binding is the binding of low con-

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centrations of radioactive ligand that is abolished by higher concentrations of nonradioactive ligand. The data are expressed in the figures as a percentage of maximum specific binding relative to a saturating concentration of atrazine. The photosystem II-mediated reduction of dichlorophenolindophenol (DCPIP) was measured at 580 nm using a Hewlett-Packard Model 8450A UV/VIS Spectrophotometer equipped with a spectrophotometer-controlled actinic source [7].

The preparation of the optically active triazines and ureas was carried out using commercially available optically active amines. For the triazines, the appropriate dichloro N-alkylaminotriazine was treated with the requisite optically active amine in methylene chloride or tetrahydrofuran with an equivalent of diisopropylethylamine. The ureas were synthesized by treating the appropriate aryl isocyanate with the requisite optically active amine in tetrahydrofuran or methylene chloride in the presence of a catalytic amount of diisopropylethylamine. The triazine and urea products were purified by crystallization, and the structures and purity were determined by NMR, mass spectrometry, and elemental analysis.

The optical rotations were obtained in either chloroform or absolute ethanol as 1% (w/v) solutions at 589 nm on a Perkin-Elmer Model 241 MC Polarimeter.

Results

α-Methylbenzyl triazines

The binding activities of the optically active N′-ethyl triazines are compared with the racemate and with atrazine in Fig. 1. The *S*(−)-isomer (compound **2**) is much more active than the *R*(+)-isomer (compound **3**), whereas the racemate (compound **1**) falls between them and shows activity comparable to atrazine. A similar structure-activity relationship is seen for inhibition of photosynthetic electron transport in Fig. 2. Again, the *S*(−)-isomer was more active than the *R*(+), and the racemate and atrazine fell in between. The activities of these compounds are quantitatively compared in the two assay systems in Table I. The concentration of the ligand necessary to displace 50% of the specific [^{14}C]atrazine binding is compared with that necessary to inhibit the rate of photosynthetic electron transport by 50%. As expected from previous work [6], the behavior of the compounds in the two *in vitro* systems is quite similar, and the less active isomer requires an approximately 15-fold higher concentration to achieve the 50% effect than does the more active isomer.

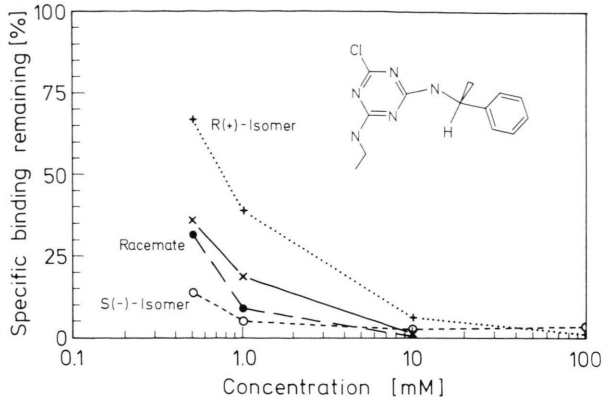


Fig. 1. Competition for atrazine binding sites by optical isomers of the α-methylbenzyl analogue of atrazine. [^{14}C]Atrazine was added to pea chloroplasts at 0.1 μM, along with solvent (methanol) or non-radioactive compound at the concentration indicated. Specific binding is the amount of [^{14}C]atrazine displaced by the non-radioactive compound. The activity of atrazine is indicated by the solid line.

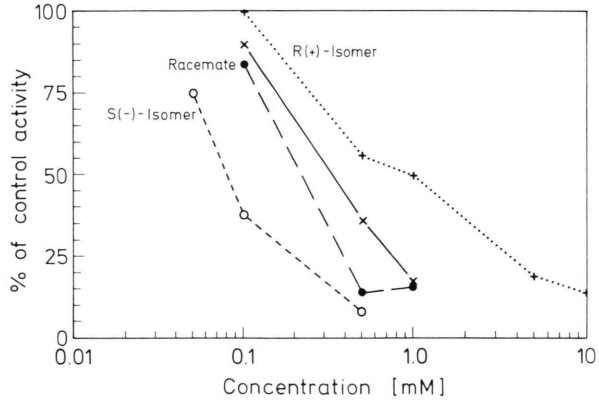
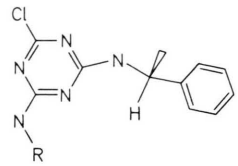


Fig. 2. Inhibition of photosynthetic electron transport by optical isomers of the α-methylbenzyl analogue of atrazine. The photosystem II-mediated reduction of dichlorophenolindophenol (DCPIP) was measured at 580 nm in pea chloroplast membranes at 5 μg Chl/ml. The activity of atrazine is indicated by the solid line.

A systematic attempt was made to modify the N′-alkyl substituent opposite to the chiral center and determine the consequential effects of this substitution on chiral discrimination (Table I). Activities of these compounds were consistant with known struc-

Table I. Comparative properties of triazines synthesized from α-methylbenzylamine.



Compound No.	R	$[\alpha]_D^{20}$	Binding 50% Max. [μM]	Rel. act.	Electron transport I_{50} [μM]	Rel. act.	Whole plant phytotoxicty PE	PO
1	C_2H_5	–	0.29	6	0.22	3	33	43
2	C_2H_5	−149.0	0.047	1	0.080	1	32	52
3	C_2H_5	+145.6	0.76	16	1.1	14	26	38
4	iC_3H_7	–	0.545	2			18	35
5	iC_3H_7	−141.5	0.252	1			21	42
6	iC_3H_7	+134.6	2.59	10			23	24
7	c-propyl	–	0.0459	1	0.102	2	30	50
8	c-propyl	−145.2	0.040	1	0.059	1	30	51
9	c-propyl	+143.5	0.117	3	0.195	3	23	39
10	$C(CH_3)_2CN$	–	4.30	3			24	41
11	$C(CH_3)_2CN$	−196.9	1.62	1			25	45
12	$C(CH_3)_2CN$	+198.0	50.0	31			3	16

ture-activity relationships for triazine herbicides [8], with cyclopropyl (compounds **7**–**9**) being the most active. There seems to be a weak steric effect of this substituent on the degree of chiral discrimination. The greatest degree of difference in activity between the optical isomers, about 30-fold, was seen in the compound with the bulkiest substituent, N′-methyl-cyanoethyl (compounds **10**–**12**), analogous to cyanazine.

The *in vivo* performance of these compounds is also summarized in Table I. All the triazines were quite active, and, as expected from previous studies on fluorophenyl ureas [6], there was a good correlation between the *in vitro* assays and postemergence herbicidal activity. The *S*(−)-isomer (compound **2**) was significantly more active than the *R*(+)-isomer, and the activity of the racemate fell between the two optical isomers. This relative relationship held independently of the N′-alkyl substituent.

α-Methylbenzyl phenylureas

Although the α-methylbenzyl derivatives of diuron are all less active than atrazine (and also diuron) in both *in vitro* systems, the two stereoisomers do show major differences in activity with the racemate falling in between. Competition for atrazine binding sites is shown in Fig. 3, with compound **14** having an affinity approximately 24-fold stronger than compound **15** (Table II). A similar result was seen for inhibition of photosynthetic electron transport (Fig. 4), with an 8-fold difference between the two optically active isomers. As might be expected, the more active urea

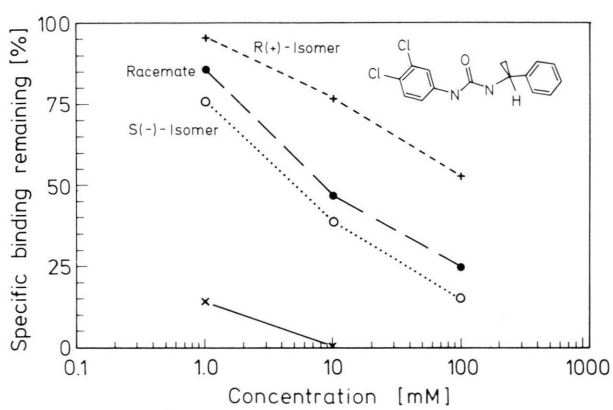
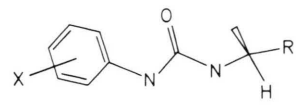


Fig. 3. Competition for atrazine binding sites by optical isomers of the α-methylbenzyl analogue of diuron. The concentration of [^{14}C]atrazine was 0.2 μM. All other details were as in Fig. 1.

Table II. Comparative properties of optical isomers of phenylureas.



Compound No.	R	X	$[\alpha]_D^{20}$	Binding 50% Max. [μM]	Rel. act.	Electron transport I_{50} [μM]	Rel. act.	Whole plant phytotoxicity PE	PO
13	phenyl	3,4-Cl_2	–	8.3	2	4.5	2	0	0
14	phenyl	3,4-Cl_2	−95.0	5.1	1	2.2	1	0	6
15	phenyl	3,4-Cl_2	+93.3	120	24	18	8	0	0
16	phenyl	2-F	–	22.7	4	117	3	21	16
17	phenyl	2-F	−54.4	5.75	1	45.2	1	32	25
18	phenyl	2-F	+54.5	> 1000	174	329	7	17	0
19	ethyl	3,4-Cl_2	–	0.275	1			4	18
20	ethyl	3,4-Cl_2	−20.1	1.53	5			3	0
21	ethyl	3,4-Cl_2	+19.8	0.290	1			11	24
22	ethyl	2-F	–	0.580	0.1			50	28
23	ethyl	2-F	−21.7	6.10	1			36	15
24	ethyl	2-F	+22.0	> 1000	164			49	34

was synthesized from the same isomer of α-methylbenzylamine as the more active triazine. These compounds did not demonstrate sufficiently high herbicidal activity to allow meaningful *in vivo* comparisons.

Compounds synthesized from chiral 2-butylamine

In the above experiments demonstrating chiral recognition in photosystem II, we have chosen substituents – methyl and phenyl – where discrimination would be maximal. In order to determine the minimum structural differences necessary for stereoselectivity, triazine and urea derivatives were synthesized from optically active 2-butylamine. For the N′-ethyl triazines, it is clear that the site shows little or no discrimination between methyl and ethyl substituents on the chiral center (compounds **26**, **27**). These compounds were essentially equivalent in their ability to compete for atrazine binding (Fig. 5) and in their postemergence phytotoxicity (Table III). Only in the case of the bulky N′-methylcyanoethyl substituent (compounds **35**, **36**) was there significant

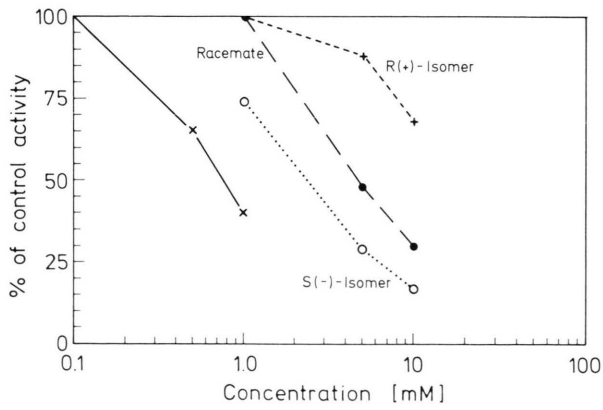


Fig. 4. Inhibition of photosynthetic electron transport by optical isomers of the α-methylbenzyl analogue of diuron. All details were as in Fig. 2.

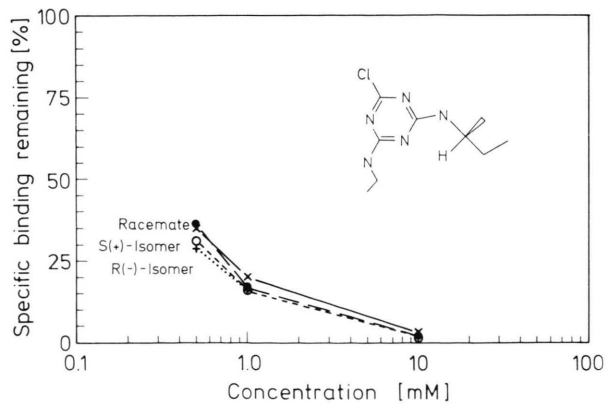
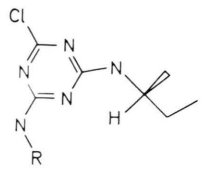


Fig. 5. Competition for atrazine binding sites by optical isomers of the N-2-butyl analogue of atrazine. All details were as in Fig. 1.

Table III. Comparative properties of triazines synthesized from 2-butylamine.



Compound No.	R	$[\alpha]_D^{20}$	Binding 50% Max. [μM]	Rel. act.	Electron transport I_{50} [μM]	Rel. act.	Whole plant phytotoxicity PE	PO
25	C_2H_5	–	0.315	1.3			39	21
26	C_2H_5	−30.1	0.239	1.0			36	28
27	C_2H_5	+28.8	0.280	1.2			40	28
28	iC_3H_7	–	0.439	1.4			13	24
29	iC_3H_7	−27.1	0.455	1.4			23	21
30	iC_3H_7	+28.0	0.318	1.0			23	23
31	c-propyl	–	0.770	1.4	0.107	0.8	38	35
32	c-propyl	−28.8	0.675	1.2	0.140	1.0	37	32
33	c-propyl	+29.2	0.560	1.0	0.135	1.0	39	38
34	$C(CH_3)_2CN$	–	2.91	6.5			24	25
35	$C(CH_3)_2CN$	−19.3	0.445	1.0			30	28
36	$C(CH_3)_2CN$	+20.0	3.35	7.5			19	16

discrimination between the optical isomers of triazines derived from chiral 2-butylamine.

The N-2-butyl derivatives of diuron do show about a 5-fold difference in activity between the optical isomers (Table II). The corresponding derivatives of 2-fluorophenyl urea (compounds **23**, **24**) show an even greater degree of discrimination, especially in the binding assay, where the *S*(+)-isomer was essentially inactive at 1 mM.

N-Methylcyclohexylmethyl atrazine

Steric factors clearly play a critical role in the degree of chiral discrimination at the photosystem II site. However, are electronic factors also important at this position in the molecule? Atrazine derivatives were synthesized from chiral 1-cyclohexylethylamine in order to probe this question. The molecules were quite active, with the *S*(+)-isomer (compound **39**) about equivalent to atrazine (Table IV). There was about an 8-fold difference between the isomers in affinity for the atrazine binding site (Fig. 6), with the racemate approximately equivalent in activity to the more active *R*(−)-isomer (compound **38**). This discrimination was also apparent in whole-plant phytotoxicity, although it was not as pronounced in electron transport (Table IV). Thus, the chirality-related differences of the α-methylbenzyl triazines (compounds **2** and **3**) observed both *in vitro* and *in vivo* are also seen in the saturated derivatives **38** and **39**. This implies that the phenyl ring with its planar geometry and potential π interaction is not a requisite for the chiral discrimination observed in the biological activity of these triazines.

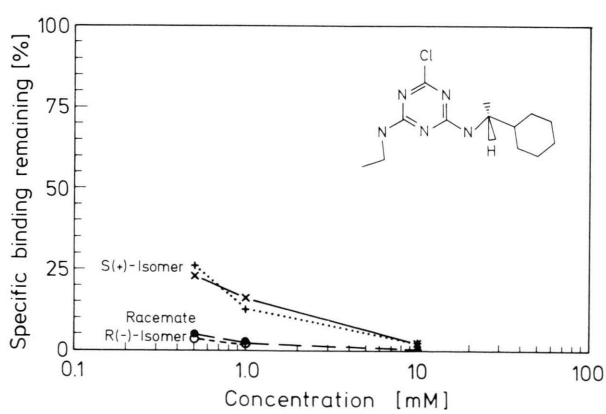
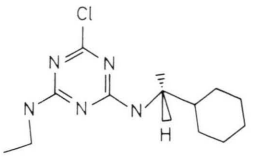


Fig. 6. Competition for atrazine binding sites by optical isomers of the N-methylcyclohexylmethyl analogue of atrazine. All details were as in Fig. 1.

Table IV. Comparative properties of triazines synthesized from 1-cyclohexylethylamine.



Compound No.	$[\alpha]_D^{20}$	Binding 50% Max. [μM]	Rel. act.	Electron transport I_{50} [μM]	Rel. act.	Whole plant phytotoxicity PE	PO
37	–	0.0409	1.2	0.0171	0.4	16	42
38	−45.0	0.0328	1.0	0.0441	1.0	21	44
39	+26.4	0.258	7.9	0.090	2.0	0	10

Discussion

These experiments clearly demonstrate that chiral recognition is an important contributor to the affinity and activity of photosystem II herbicides. Steric factors play a major role in this degree of chiral discrimination since differences in activity are greatly reduced if the chiral substituents are methyl and ethyl rather than methyl and phenyl. In the optically active triazines, bulky N-alkyl substituents on the opposite side of the molecule increase the degree of stereoselectivity. Electronic factors probably contribute little to this discrimination, since the analogues in which the chiral substituents are methyl and cyclohexyl still show substantial differences in activity.

In the only report on photosystem II chirality prior to the inception of this work, Moreland and Boots [4] synthesized compounds **13**, **14**, and **15**. They found similar effects on photosynthetic ferricyanide reduction as those seen here in Fig. 4, with the *S*(−)-isomer substantially more active than the *R*(+)-isomer, and a significant reduction in activity compared to diuron. Since the present work was begun, Phillips and Huppatz [9] used optically active α-methylbenzyl amine to synthesize optically active isomers of cyanoacrylate inhibitors of PS II. In that example, there was a 200-fold difference in activity between the *R*- and the *S*-isomers. At this time it is not clear how these molecules relate to the triazines and ureas.

It will be important to map the structures of various classes of PS II herbicides to determine the number and location of different potential chiral centers that may have physiological importance. The common structural feature for this type of photosynthetic inhibitor is an N−H group attached to an electron-deficient sp^2 carbon atom [3]. In the triazine **2** the assymetric carbon is adjacent to this essential N−H. However, in the urea the essential N−H is that adjacent to the substituted phenyl ring, and thus the assymetric carbon of compound **14** is two atoms removed from the essential N−H. Although these triazine and urea molecules have spatially different chiral centers, the absolute stereoselectivity at each center is similar since the more active isomer of each pair derives from the same starting material.

Another line of reasoning also argues that the chiral center of these triazines must occupy a different position in space than that of the ureas. The degree of chiral discrimination was about the same in the two classes. However, the α-methylbenzyl substitution had little effect on the absolute activity of the triazines but reduced the urea activity by two orders of magnitude. The chiral center in the cyanoacrylates [9] must occupy a third position, since the same chiral substituents show a 200-fold difference in activity rather than 10−20-fold.

It is premature to speculate as to whether the stereoselectivity shown by photosystem II herbicides is purely a fortuitous reflection of a proteinaceous binding site or whether the chirality-recognition regions of the receptor play a role in electron transport or in binding of the endogenous ligand, plastoquinone. Resolution of this question will require a

detailed stereochemical model of the binding site in the PS II reaction center. In the meantime, structure-activity relationships such as those described here help to define essential structural features that such a stereochemical model must encompass.

Acknowledgements

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