Cyanoacrylate Inhibitors of Photosynthetic Electron Transport. Nature of the Interaction with the Receptor Site

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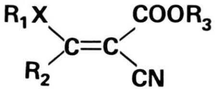
The inhibitory activities (pI_{50} values) of a series of 3-alkylamino-2-cyanoacrylic acid ester derivatives in relation to photosynthetic electron transport in isolated pea chloroplast suspensions are reported. Assuming such pI_{50} values reflect the relative binding affinities for the inhibitor receptor site, the results suggest that several groups in these molecules interact specifically with the binding domain so that a multi-point attachment is formed. Stereochemical factors appear to play a significant role in the interaction.

Introduction

Herbicidally active compounds which interfere with photosynthetic electron transport (PET) in the vicinity of photosystem II (PS II) have generally been classified as amide (*e.g.* propanil, diuron, bromacil, atrazine, metribuzin) or phenol (*e.g.* DNOC, dinoseb, ioxynil) type derivatives [1, 2]. The interaction of these herbicides with the thylakoid membrane has been shown to be reversible, and competitive displacement studies indicate that they share a common binding area at the membrane-protein interface [3–7]. It has been proposed that in amide type inhibitors the electron deficient sp_2 carbon adjacent to a nitrogen atom with a lone pair of electrons is the essential structural element involved in their interaction with the receptor site [1, 2].



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Recently, a new class of PS II PET inhibitors have been recognized, *viz.,* 3-N-methylanilino-2-cyanoacrylic acid ester derivatives (**I,** R_1 = phenyl; X = NCH_3; R_2 = H; R_3 = alkyl) [8]. These compounds behave as typical PS II inhibitors in competitively displacing metribuzin from the membrane binding site [9]. They cannot be regarded as amide or phenol derivatives although they have some chemical analogy to the amides in being vinylogous carbamates.

Further studies [10, 11] have shown that PET inhibitory activity is retained when the arylamino group is replaced by an alkylamino moiety to give lipid-like molecules (**I,** $R_1 R_3$ = alkyl; X = NH; R_2 = H), which not only lack the amide or phenol group but also differ from other PET inhibitors in not possessing an aryl function. These lipid-like PET inhibitors have proved particularly useful in probing the nature of the binding domain [12].

Results and Discussion

Table I records pI_{50} values for inhibition of the Hill reaction rate in pea chloroplasts fragment for a series of 3-alkylamino-2-cyanoacrylic acid ester derivatives related to structure **I.**

Optimum inhibitory activity is associated with an asymmetrical distribution of alkyl group sizes in the molecule implying a corresponding asymmetry in the distribution of lipophilic areas around the binding domain. This is indicated by pI_{50} data for the series of compounds **1–5** in which R_1 and R_3 are varied but the total number of methylene groups in $R_1 + R_3$ is kept constant. Moreover, pI_{50} values are similar whether the longer alkyl chain is associated with the amino (R_1) or ester (R_3) function (*cv.* **1 & 5**; **2 & 4**), suggesting that any

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Abbreviations: Bromacil, 5-bromo-3-s-butyl-6-methyluracil; dinoseb, 2,4-dinitro-6-*sec*-butylphenol; diuron, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; DNOC, 4,6-dinitro-*o*-cresol; ioxynil, 4-hydroxy-3,5-diiodobenzonitrile; metribuzin, 4-amino-6-isopropyl-3-methyl-thio-1,2,4-triazin-5-one; PET, photosynthetic electron transport; PS, photosystem; propanil,3,4-dichloropropionanilide.

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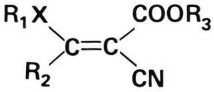
Table I. PET inhibitory activity of 3-alkylamino-2-cyanoacrylic acid ester derivatives.



Compd. No.	R_1	X	R_2	R_3	pI_{50}	Ref.
1	C_2H_5	NH	H	$C_{10}H_{21}$	5.0	10
2	C_4H_9	NH	H	C_8H_{17}	4.1	10
3	C_6H_{13}	NH	H	C_6H_{13}	3.6	10
4	C_8H_{17}	NH	H	C_4H_9	4.2	10
5	$C_{10}H_{21}$	NH	H	C_2H_5	5.1	10
6	$C_{10}H_{21}$	NH	H	CH_3	5.2	10
7	CH_3	NH	H	$C_{10}H_{21}$	4.9	10
8	$C_{10}H_{21}$	NH	C_2H_5	CH_3	6.2	15
9	CH_3	NH	C_2H_5	$C_{10}H_{21}$	4.0	15
10	$C_{10}H_{21}$	NH	H	C_5H_{11}	< 3.4	11
11	$C_{10}H_{21}$	NH	H	$(CH_2)_2OC_2H_5$	7.3	11
12	$C_{10}H_{21}$	NH	H	C_6H_{13}	< 3.4	15
13	$C_{10}H_{21}$	NH	H	$(CH_2)_2OC_3H_7$	6.5	11
14	$C_{10}H_{21}$	NH	H	$(CH_2)_3OC_2H_5$	4.8	11
15	C_4H_9	NH	H	$(CH_2)_2OC_2H_5$	4.5	11
16	C_6H_{13}	NH	H	$(CH_2)_2OC_2H_5$	4.5	11
17	C_8H_{17}	NH	H	$(CH_2)_2OC_2H_5$	6.4	11
18	C_8H_{17}	NCH_3	H	$(CH_2)_2OC_2H_5$	5.1	13
19	C_8H_{17}	S	H	$(CH_2)_2OC_2H_5$	5.7	13
20	C_8H_{17}	O	H	$(CH_2)_2OC_2H_5$	4.2	13
21	C_8H_{17}	CH_2	H	$(CH_2)_2OC_2H_5$	4.5	13
22	C_8H_{17}	NH	CH_3	$(CH_2)_2OC_2H_5$	6.6	13
23	C_8H_{17}	NH	C_2H_5	$(CH_2)_2OC_2H_5$	7.1	13
24	C_8H_{17}	NH	C_3H_7	$(CH_2)_2OC_2H_5$	6.6	13
25	C_8H_{17}	NH	C_4H_9	$(CH_2)_2OC_2H_5$	5.5	13
26	$S(+)C_6H_5CH(CH_3)$	NH	C_2H_5	$(CH_2)_2OC_2H_5$	7.1	15
27	$R(-)C_6H_5CH(CH_3)$	NH	C_2H_5	$(CH_2)_2OC_2H_5$	4.8	15

interacting group or groups in the cyanoacrylate moiety must be symmetrically placed. It is likely that both the nitrile and ester carbonyl functions are involved in the inhibitor/receptor site interaction since removal of either the nitrile or ester group leads to almost complete loss of inhibitory activity [15]. The results would therefore suggest that both the nitrile and ester carbonyl functions must be able to occupy the same sites in the binding domain irrespective of which way round the molecule is oriented with respect to the alkyl groups. Introduction of an alkyl group into the 3-position presumably disturbs the symmetry of the molecule and results in the pI_{50} value being significantly greater when the longer alkyl chain is attached to the amino rather than to the ester group (*cv.* **8** & **9**), the corresponding unsubstituted compounds **6, 7** having intermediate values. This points to a positive interaction between the 3-ethyl group and the receptor site in molecules in which the more lipophilic alkyl group is attached to the amino function.

Insertion of an ether oxygen into the alkyl ester chain three atoms removed from the carbonyl function enhances activity by from 1000 to 10 000 fold (**10** & **11**, **12** & **13**) whilst shifting the oxygen one atom further away from the ester carbonyl reduces activity 50 fold (**13** & **14**). This points to a highly localised hydrophilic pocket being located in an otherwise lipophilic environment close to where the carbonyl interacts with the receptor site.

The ethoxyethyl esters have proved a particularly useful series for studying the effect of structural changes on the activity of these molecules. Thus the effect of increasing the chain length of the 3-alkylamino group on inhibitory activity (~ 0.5 pI_{50} units per methylene group *cv.* **11, 15, 16, 17**) is similar to its effect on the logarithm of the octanol-water partition coefficient (P) (~ 0.5 log P units per methylene group [14]) and suggests that the 3-alkylamino group is interacting with an essentially fluid hydrocarbon phase similar to the interior of the lipid membrane.

When the amino group in the 3-octylamino ethoxyethyl ester series is replaced by a sulphur, oxygen, methylamino or methylene group inhibitory activity is reduced but not lost (**17**–**21**) indicating that activity is associated not only with vinylogous carbamates but also with vinylogous carbonates and thiocarbonates and unsaturated acids in which no vinylogous amide function is present. Surprisingly, however, there is no obvious correlation between the electron donating power of the X group, as measured by its effect on the carbonyl stretching frequency in the infra-red ($NMe > NH > S > O > CH_2$) [13] and its effect on pI_{50} ($NH > S > NCH_3 > CH_2 > O$). Electronic effects might have been expected to influence the affinity of both the carbonyl and nitrile groups for the binding site but stereochemical considerations discussed below may well have obscured such trends.

3-Alkyl-3-octylamino ethoxyethyl esters **22**–**25** show a parabolic relationship between pI_{50} and alkyl chain length with optimum activity associated with the 3-ethyl derivative **23**. This implies a marked degree of spatial specificity in the interaction between the 3-alkyl group (R_2) and the receptor site.

The introduction of alkyl groups into the 3-position influence the stereochemistry of the molecule by favouring the geometrical isomer in which the X and ester functions occupy a *cis* configuration [13]. Since most of the potent alkylamino cyanoacrylate inhibitors of the Hill reaction rate tend to exist in the *cis* form it has been speculated that such isomers conform more closely to the structural requirements of the receptor site than their *trans* analogues [13]. This could account for the poor correlation observed between electronic influences and inhibitory activity of the different X derivatives discussed above **17**–**21** since those which favour a *cis* configuration, *viz.*, the amino and thio-compounds **17** & **19** are more active than would have been predicted on the basis of electronic effects whilst those which favour a *trans* configuration, *viz.*, the methylamino and oxy-compounds **18** & **20** are less active.

A highly specific inhibitor-receptor site interaction is indicated by the 200 fold difference in activity between the R(−) and S(+) isomers of the 3-α-methylbenzylamino derivative **26, 27**. This represents a relatively high level of discrimination between the optical isomers and points clearly to the involvement of a protein · chiral centre in the interaction.

The complexity of the binding of cyanoacrylate inhibitors to the thylakoid membrane is reflected in the relationship between pI_{50} and structure. The lipophilic balance within the molecule as determined by the amino and ester alkyl groups (R_1, R_3) as well as its overall spatial configuration as determined by *cis-trans* isomerism plays a significant role in the interaction. In addition, groups within the molecule appear to interact individually with discrete sites in the binding domain. Some of these interactions such as the hydrophobic type bonding involving the alkylamino chain (R_1) are relatively non-specific. Others, however, involving, for example, the R_2 alkyl group, the ether oxygen in the alkoxyester chain (R_3) and the α-methyl group in the optically active 3-α-methylbenzylamino isomers **26, 27** are highly specific and emphasize the importance of stereochemical factors.

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[1] K. H. Buchel, Pestic. Sci. **3**, 89 (1972).
[2] A. Trebst and W. Draber, in Advances in Pesticide Chemistry (H. Geissbühler, ed.), Part 2, pp. 223–234, Pergamon Press, New York 1979.
[3] K. Pfister and C. J. Arntzen, Z. Naturforsch. **34c**, 996 (1979).
[4] W. Tischer and H. Strotmann, Biochim. Biophys. Acta **460**, 113 (1977).
[5] S. Reimer, K. Link, and A. Trebst, Z. Naturforsch. **34c**, 419 (1979).
[6] K. E. Pallet and A. D. Dodge, Pestic. Sci. **10**, 216 (1979).
[7] W. Oettmeier, K. Masson, and U. Johanningmeier, Biochim. Biophys. Acta **679**, 376 (1982).
[8] J. L. Huppatz, J. N. Phillips, and B. M. Rattigan, Agric. Biol. Chem. **45**, 2769 (1981).
[9] A. Trebst, personal communication.
[10] J. N. Phillips and J. L. Huppatz, Agric. Biol. Chem. **48**, 51 (1984).
[11] J. N. Phillips and J. L. Huppatz, Agric. Biol. Chem. **48**, 55 (1984).
[12] J. N. Phillips and J. L. Huppatz, Proc. 6th Int. Cong. Photosynthesis, in press (1983).
[13] J. L. Huppatz and J. N. Phillips, Z. Naturforsch., submitted 1983.
[14] C. Hansch, A. Leo, S. H. Unger, K. H. Kim, D. Nikaitani, and E. J. Lien, J. Med. Chem. **16**, 1207 (1973).
[15] Unpublished observations.