Cyanoacrylate Inhibitors of the Hill Reaction
III. Stereochemical and Electronic Aspects of Inhibitor Binding

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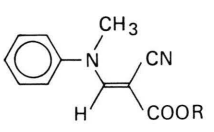
Ethoxyethyl 3-octylamino-2-cyanoacrylate and related compounds in which the amino group was replaced by $N-CH_3$, S, O and CH_2 were synthesized and their activity as inhibitors of the Hill reaction in isolated pea chloroplasts determined. All compounds showed moderate to high activity but there was no obvious correlation between activity and the electronic character of the ester carbonyl group. The stereochemistry of the various inhibitor molecules was deduced from the PMR spectra and the possible influence of stereochemistry on Hill inhibitory activity discussed. Replacement of the olefinic proton in the 2-cyanoacrylates with a β-alkyl substituent was examined and a specific relationship between the length of the alkyl chain and activity was observed.

Introduction

The inhibition of photosynthetic electron transport in the vicinity of photosystem II (the Hill reaction) is a property common to a number of the major classes of commercial herbicides. Such compounds have generally been classified as either amide-type inhibitors, such as the phenylamides (*e.g.* propanil), phenylureas (diuron), uracils (bromacil), pyridazinones (pyrazon) and triazinones (metribuzin), or phenol-type compounds, such as DNOC and bromoxynil [1].



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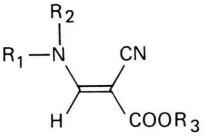

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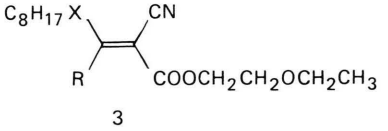

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Recently, we reported a new group of photosynthetic electron transport inhibitors [2], the 2-cyanoacrylates (**1**), which, although they appear to act at the same receptor site as other photosystem II inhibitors [3], cannot be classified as being of either the amide- or phenol-type. Modification of structure **1** by replacement of the phenylamino substituent with long-chain alkylamino groups gave lipid-like inhibitor molecules which have proved useful in probing the nature of the inhibitor receptor site [4]. The introduction of an ether linkage into the ester side-chain (*e.g.* **2**, $R_1 = C_{10}H_{21}$; $R_2 = H$; $R_3 = CH_2CH_2OCH_2CH_3$) was particularly beneficial and produced compounds of high intrinsic Hill inhibitory activity [5]. A number of inferences concerning the possible binding domains of these compounds were drawn from the structural requirements for Hill inhibition [4, 5]. In particular, the results suggested the possibility of a single specific centre of interaction with the inhibitor receptor site [4]. A possible candidate for such an interaction in the cyanoacrylate molecule is the carbonyl group, particularly as it is a potential hydrogen bond acceptor and hydrogen bonds are thought to be implicated in inhibitor binding [6, 7]. Accordingly, we have synthesized molecules in which the alkylamino residue has been replaced by substituents expected to modify the electronic character of the carbonyl group and examined their activity as inhibitors of the Hill reaction in isolated pea chloroplast fragments.

In earlier work with compounds of general formula **2**, certain derivatives were found to be mixtures of stereoisomers by examination of their PMR spectra. This suggested the possibility that biological activity may be dependent on the stereochemistry of the molecule. The isomeric composition of a series of compounds in which the olefinic proton attached to the acrylate double bond was replaced by a β-alkyl group was therefore examined

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in an attempt to assess the importance of the spatial configuration of groups attached to the central double bond in determining inhibitory activity. To enable evaluation of electronic and stereochemical effects to be undertaken in a comparable series, all compounds synthesized were based on structure **3**, which incorporated a C_8 straight-chain alkyl group and an ethoxyethyl ester substituent.

Materials and Methods

Synthesis of compounds

All compounds recorded in Tables 1 and 2 gave analytical data within satisfactory limits and the structures were confirmed by the PMR spectra which were recorded on a Jeol FX90Q spectrometer using TMS as internal standard and $CDCl_3$ as solvent. Infrared spectra were recorded on a Pye Unicam SP3-200 spectrophotometer in chloroform solution.

Compound **4** was reported previously [5]. The PMR spectrum of a freshly prepared sample showed signals at δ 7.97 and 7.79 (J = 15.5 Hz) and 7.38 and 7.23 (J = 14 Hz) for the olefinic proton and two broad signals at δ 9.00 and 6.52 for the NH group. After deuteration, the latter signals disappeared and the doublets due to the olefinic proton collapsed to two singlets at δ 7.87 and 7.31 in the ratio 1:2 (integration). The spectrum of a sample which had been allowed to stand for several months showed the olefinic proton as a doublet at δ 7.97 and 7.79 (J = 15.5 Hz) and the NH group as a broad signal at δ 6.52. After deuteration, the NH signal disappeared and the olefinic proton appeared as a singlet at δ 7.87.

Compound **5** was prepared from compound **4** by methylation under phase transfer conditions [4]. The remaining amino compounds **10–12** were prepared from the corresponding ethoxy or methoxy acrylic acid derivatives by reaction with octylamine at 100–120° for 30 min [4].

The thioesters **6**, **13–16** and ethers **7**, **17–20** were obtained similarly using 1-octanethiol and 1-octanol. However, more vigorous conditions (180–190° for two hours) were required.

The ethoxy or methoxy acrylic acid derivatives were prepared by reacting ethoxyethyl cyanoacetate with triethyl orthoacetate, triethyl orthopropionate, trimethyl orthobutyrate and trimethyl orthovalerate, respectively, in the presence of acetic anhydride at 120–130° [2]. The boiling points and percentage yields are recorded below: ethoxyethyl 3-ethoxy-2-cyanocrotonate, 141–143°/0.1 mm, m.p. 43–45°, 56%; ethoxyethyl 3-ethoxy-3-ethyl-2-cyanoacrylate, 125–126°/0.05 mm, 54%; ethoxyethyl 3-methoxy-3-propyl-2-cyanoacrylate, 150–152°/0.3 mm, 48%; ethoxyethyl 3-methoxy-3-butyl-2-cyanoacrylate, 131–134°/0.01 mm, 46%.

Ethoxyethyl cyanoacetate (5.2 g) and decyl aldehyde (5.2 g) were dissolved in toluene (100 ml) and treated with piperidine (0.2 ml) in glacial acetic acid (2 ml). The mixture was boiled under reflux and the water formed was separated. When the reaction was complete, the toluene solution was washed with water, dried and the solvent removed *in vacuo*. The residue was distilled to give *ethoxyethyl 2-cyano-2-dodecenoate* (**8**) (6.8 g, 70%) as a colourless liquid, b.p. 157–159°/0.2 mm. This compound slowly decomposed on storage with consequent loss of Hill inhibitory activity. *Ethoxyethyl 2-cyano-3-methyl-2-dodecenoate* (**21**) was prepared similarly from ethoxyethyl cyanoacetate and 2-undecanone. It was obtained as a colourless liquid (96% yield), b.p. 152–153°/0.1 mm.

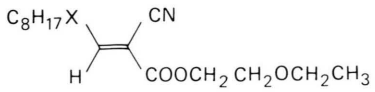
Hill reaction assay

Compounds were assayed for Hill inhibition activity using chloroplast fragments isolated from the leaves of 21 day-old plants of *Pisum sativum* (c.v. Victory Freezer), the electron acceptor being the indicator dye 2,3′,6-trichlorophenolindophenol. The experimental procedure was as described elsewhere [8]. The activity of a compound as a Hill inhibitor was expressed in terms of its pI_{50} value, *i.e.* $-\log_{10} I_{50}$, where I_{50} was the molar concentration required to decrease the rate of dye reduction under illumination of saturating intensity to 50% that obtained in the absence of the compound.

Results and Discussion

The compounds recorded in Table I were assayed as inhibitors of the Hill reaction and the pI_{50} values reflect the effect of replacing the NH group in compound **4** with $N-CH_3$, S, O and CH_2 (compounds **5–8**, respectively). The carbonyl and nitrile infrared stretching frequencies, together with the PMR chemical shift of the olefinic proton are also recorded in Table I.

Table I. Physical data and pI_{50} values for compounds of general formula **3** (R=H).



Compd. no.	X	b.p. [mm]	δ_{CH} [ppm]	$\lambda_{C=O}$ [cm^{-1}]	$\lambda_{C\equiv N}$ [cm^{-1}]	pI_{50}
4	NH	200–201 (0.05)	7.87[a]	1703	2212	6.40
5	N–CH_3	202–204 (0.2)	7.71	1696	2212	5.10
6	S	183–185 (0.05)	8.52 8.10[b]	1721	2228	5.70
7	O	165–166 (0.01)	8.02	1724	2240	4.20
8	CH_2	146–148 (0.05)	7.67 (t)	1734	2230	4.50

[a] After deuteration in the spectrum of the *E*-isomer.
[b] This signal accounts for approximately 10% of the total due to the olefinic proton.

As inhibitors of photosynthetic electron transport, all compounds in Table I were at least moderately effective, though activity ranged over one hundred fold (*c.f.* compounds **4** and **7**). A notable feature of the results was the relatively weak activity of the N–CH_3 compound **5** when compared with the analogous NH compound **4**. In earlier studies [4] with alkyl esters, activity remained virtually unchanged whether an NH or N–CH_3 group was present in the molecule, *e.g.* **2**, $R_1 = C_8H_{17}$; R_2 = H; $R_3 = CH_3$; pI_{50} 4.7, *cf.* **2**, $R_1 = C_8H_{17}$; $R_2 = CH_3$; $R_3 = CH_3$; pI_{50} 4.6. This would suggest that the normally advantageous ethoxyethyl ester residue was less able to exert a favourable influence on activity in the case of the N–CH_3 compound **5**, perhaps because of the stereochemical orientation of the molecule (*vide infra*).

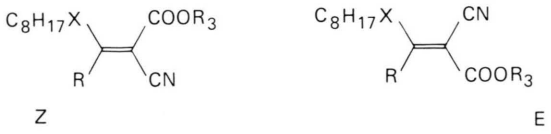
The retention of moderate activity in compound **8** where the left-hand side of the molecule consisted solely of an alkyl chain was somewhat surprising. This result was particularly difficult to reconcile with the generally accepted concept of the structural features necessary for activity in the many chemical families which inhibit PS II electron transport. In particular, the amide-type inhibitors are considered to possess a common "essential element" (an electron deficient sp^2-carbon adjacent to a nitrogen atom with a lone pair of electrons) together with specificity-determining hydrophobic substituents [1, 9]. While it may be reasonable to reconcile compounds **4** and **5** with this model on the grounds that they may be considered vinylogous carbamates, the remaining compounds **6**–**8** do not conform to the "essential element" hypothesis.

The possibility of the ester carbonyl group being involved in specific binding to the receptor site is worthy of consideration, particularly as this group would be expected to act as a hydrogen bond acceptor and may contribute to inhibitor binding [6, 7]. If the carbonyl moiety was involved in binding at the receptor site in this fashion, it would be reasonable to expect a correlation between inhibition of electron transport and the carbonyl stretching frequency of the molecule in the infrared spectrum. The data presented in Table I does not reveal any obvious correlation between the carbonyl stretching frequency and biological activity as represented by the pI_{50} values, even though the nature of X profoundly affected both parameters. The nitrile stretching frequencies provided a somewhat better correlation (with the exception of compound **5**) but the range of values was too narrow to allow any worthwhile conclusions to be drawn.

These results suggest that hydrogen bonding is not a primary binding force, but it is noteworthy that the data presented in Table I refers to compounds having essentially *trans* stereochemistry* and it is possible that the stereochemical considerations discussed below may have obscured any influence of the electronic effect of the X group. Nevertheless, the data presented in Table II showing the variation in pI_{50} value with increasing chain length of the β-alkyl group does point to a highly specific interaction which may well prove important in inhibitor

* The term *trans* as used in the text refers to a *trans* arrangement of the octylamino, octylthioether, octylether, and nonyl groups with the ethoxyethyl ester.

Table II. Physical data, stereochemical assignments and pI_{50} values for compounds of general formula **3**.



$R_3 = CH_2CH_2OCH_2CH_3$

Compd. no.	X	R	b. p. [mm]	pI_{50}	Prod. ratio *E*	Isomer [%][a] *Z*
4	NH	H		6.40	33	67[b]
9		CH_3	186–188 (0.05)	6.65	–	100
10		C_2H_5	193–194 (0.05)	7.15	–	100
11		C_3H_7	191–192 (0.01)	6.60	–	100
12		C_4H_9	195–197 (0.01)	5.50	–	100
6	S	H		5.70	90	10
13		CH_3	186–188 (0.1)	5.50	45	55
14		C_2H_5	176–178 (0.01)	5.95	–	100
15		C_3H_7	196–198 (0.1)	5.40	–	100
16		C_4H_9	198–200 (0.1)	< 3.4	–	100
7	O	H		4.20	100	–
17		CH_3	170–172 (0.05)	4.40	85	15
18		C_2H_5	184–186 (0.2)	4.65	85	15
19		C_3H_7	185–187 (0.2)	4.25	85	15
20		C_4H_9	187–189 (0.1)	< 3.4	80	20
8	CH_2	H		4.50	100	–
21		CH_3	152–153 (0.1)	5.20	50	50

[a] Product isomer ratio assigned on the basis of PMR spectra ($CDCl_3$).
[b] Isomer distribution of a freshly-prepared sample. On standing several months, the material reverts completely to the *E*-isomer.

binding. The replacement of the olefinic proton in compound **4** with a β-alkyl substituent of increasing carbon chain length (compounds **9**–**12**) resulted in an increase in activity to C_2 (compound **10**) followed by a decline through C_3 to C_4 (compounds **11** and **12**). While the increase in pI_{50} values was no more than would be expected from the effect of a methylene group on the logarithm of the *n*-octanol-water partition coefficient [10], the decline in activity with further increase in lipophilicity pointed to a possible discontinuity in the region of the binding domain occupied by the β-alkyl substituent. A pocket imposing specific size constraints on the interacting group is a possible explanation for the observed parabolic effect.

The trend to maximum inhibitor activity of the β-ethyl compounds was evident in each series (Table II) although insufficient data was available to adequately define the effect in the hydrocarbon series (compounds **8** and **21**). In the thioether series, compound **13** appeared to be anomalous and an explanation for the lower than expected activity of this compound was not immediately apparent.

Stereochemistry

The stereochemistry of compound **4** was deduced from the PMR spectrum, which showed that a freshly-prepared sample consisted of a mixture of *E* and *Z* isomers in the ratio of 1 : 2. However, after several months in storage, compound **4** reverted completely to the *E*-isomer as evidenced by the appearance (after deuteration) of only the downfield signal at δ 7.87. Assignments were made on the assumption that the deshielding effect of the ester carbonyl group on the olefinic proton will result in that signal appearing further downfield in the *E*-isomer.

Compounds **9**–**12** on the other hand, were obtained in only one isomeric form (Table II), assigned the *Z* configuration on the basis of the appearance

of the NH signal well downfield (δ 9.85–9.91) with respect to that in the *E*-isomer of compound **4** (δ 6.52). The corresponding signal for the *Z*-isomer of compound **4** appeared at δ 9.0.

The problem of geometric isomerism in β-amino-β-alkyl-cyanoacrylic esters has been considered previously [11]. PMR evidence, based particularly on the presence of the NH proton in the highly deshielded region, δ 9.20–10.02, indicated that these compounds existed exclusively as chelated enamines. The *cis* form was assumed to be stabilized by intramolecular hydrogen bonding between the amino group and the ester carbonyl. The assignments made on this basis were consistent with earlier work in which ethyl β-benzylaminocrotonate was shown to exist predominantly in the chelated enamine form [12]. α,β-Unsaturated aminoketones behave similarly [12–14]. Moreover, β-alkylcyanoacrylic esters containing a β-NH_2 group showed two broadened signals due to the NH protons in the δ 9.20–10.02 and δ 5.85–6.10 ranges. The latter signals were attributed to non-hydrogen bonded NH protons. The proximity of the NH signal (δ 6.52) in the *E*-isomer of compound **4** to this region would indicate a trans-relationship of alkylamino and ester functions.

The obvious thermodynamic stability of the *cis* isomer in the case of compounds **9–12** was reversed in compound **4**, where the initial formation of a mixture of geometric isomers, albeit favouring the *cis* form, was followed by complete reversion to the *trans* form. It would appear, therefore, that the stabilisation of the *cis* arrangement of alkylamino and ester groups which might be expected from intramolecular hydrogen bonding can determine the stereochemistry of the molecule only when assisted by the steric effect introduced by replacing the olefinic proton by a β-alkyl substituent.

The stereochemistry of compounds of general formula **1** has been discussed previously [2], and, on the basis of available evidence [15, 16], compounds of that series were assumed to have a *trans* arrangement of arylamino and ester groups. The PMR spectrum of compound **5** showed the olefinic proton in the deshielded region similar in chemical shift to the olefinic proton of the *E*-isomer of compound **4**. Thus, compound **5** is also in the *trans* configuration.

The situation with the thio derivatives, **6**, **13–16** (Table II) was similar in that the molecules tended to adopt the *cis* configuration when a β-alkyl substituent was present. Compound **6** showed two signals due to the olefinic proton at δ 8.10 (10%) and 8.52 (90%). The downfield signal was considered to result from the deshielding effect on the olefinic proton of the adjacent ester carbonyl, indicating that compound **6** was obtained in predominantly the *E*-form. A similar argument pertains with compound **13**, which showed two methyl signals in the PMR spectrum at δ 2.70 (45%) and 2.60 (55%). The anisotropic effect of the carbonyl group would be expected to result in a difference in chemical shift between *cis* and *trans* methyl groups. Again, the downfield signal is attributable to the *E*-isomer. The chemical shift difference of 0.10 ppm was similar to that observed (0.12 ppm) between the methyl signals of the *E*- and *Z*-isomers in β-alkylthiocrotonic esters [17].

The remaining thio derivatives, **14–16**, appeared to exist in only one form, presumably the *Z*-isomer, although analysis of the spectra was complicated by the almost identical position of the β-methylene signal and that due to the methylene group attached to sulphur. That compounds **14–16** existed in the *Z*-form followed from the detailed study of the isomer distribution in β-alkylthiocrotonic esters referred to above [17]. The thermodynamic equilibrium in these compounds was shown to lie largely towards the *Z*-isomer. Furthermore, greater thermodynamic stability of the *cis* configuration might be expected in compounds **14–16** since a consequence of the steric effect of the β-alkyl substituent in these compounds would be to increase the electronic interaction between the alkylthio and carbonyl groups [17].

The ether derivatives, **7**, **17–20**, did not show such a marked tendency to adopt the *cis* configuration. The PMR spectrum of compound **7** showed the olefinic proton as a sharp singlet at δ 8.02. Because only one isomer was present, structural assignment remains uncertain. However, the behaviour of compounds **17–20** and literature evidence [18] suggested that, in the ether series, the more thermodynamically stable isomer was the *E*-form. Compound **7** was therefore assumed to be *trans* with respect to alkoxy and carbonyl groups.

The remaining ethers, **17–20**, occurred as equilibrium mixtures of the two isomeric forms with the *E*-isomer predominant in each case (see Table II). Compound **17** showed two methyl signals at 2.43 (*Z*-isomer) and 2.61 (*E*-isomer), the downfield

signal accounting for 85% of the total. The differential shift in the β-methyl protons between *cis* and *trans* isomers (0.18 ppm) was consistent with that observed (0.17 ppm) in simple β-alkoxy-β-alkylcyanoacrylic esters studied previously [18].

The *E*/*Z* ratio in compounds **18**–**20** appeared to be similar to that present in the β-methyl case (85/15). In these molecules, a *trans* arrangement of ester and alkoxy groups would seem to be preferred, regardless of the presence of a β-alkyl substituent.

The long-chain alkyl derivative **8** occurred as a single isomer, as shown by the appearance of the olefinic proton as a well-defined triplet at δ 7.67 in the PMR spectrum. This compound can be designated the *E*-isomer, since the thermodynamic stability in this case would be expected to be influenced by steric factors alone, thus favouring the *trans* arrangement of long-chain alkyl and carbonyl groups. Not unexpectedly, the β-methyl derivative, **21**, existed in the two isomeric forms with an *E*/*Z* ratio 50/50.

The effect of the stereochemistry of the inhibitor molecules on activity is not, as this stage, clear. The problem is compounded by the difficulty experienced in achieving an adequate separation of any of the isomeric mixtures described above. However, the most active compounds assayed (compounds **9**, **10** and **11**) were shown to have *cis* stereochemistry. On the other hand, assay of a freshly-prepared sample of compound **4** (*E*/*Z* ratio: 1/2) and of the pure *E* isomer (*trans*) failed to show any detectable difference in Hill inhibitory activity. It is difficult to reconcile this apparent indifference to stereochemistry with observations such as the importance of the ether linkage in the ester sidechain [5] and of the size of the β-alkyl group, which imply a marked degree of spatial specificity in achieving high inhibitory activity. The relatively low pI_{50} value of compound **5**, already referred to above, would appear to suggest imprecise orientation and this compound is unequivocally *trans*. Thus, it is tempting to speculate that the *cis* isomer conforms more closely to the requirements of the receptor site. This conclusion is reinforced by the data in Table II which indicate that *cis* orientation is preferred in the generally more active NH and S series. The behaviour of compound **4** may be rationalised in that, although the more thermodynamically stable isomer is *trans*, there is obviously a minimal energy barrier to interconversion between the two forms. Thus, the apparent indifference of the pI_{50} value to steric orientation in this molecule could well be the result of the conversion of the molecule to a preferred configuration at the receptor site. If this is indeed the case, the energy barrier to rotation about the carbon-carbon double bond may well be a determining factor in the effectiveness of the molecules as inhibitors of photosynthetic electron transport.

Acknowledgements

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