Effects of N-Aryl-N′,N′-Dialkyl-1,2-Ethanediamines on ATP Formation in Chloroplasts. QSAR of Amine Uncouplers

Gerard van den Berg * and Marjo Brandse

DUPHAR B. V. Biochemistry Department, Crop Protection Division, P. O. Box 4, 1243 ZG's-Graveland, The Netherlands

Z. Naturforsch. **39 c,** 107–114 (1984); received September 19, 1983

Arylethanediamines, Photophosphorylation, Uncouplers, Structure-Activity Relationships

The effects of the algicide PH 40-62 on photosynthetic reactions in spinach chloroplasts were studied. The compound proved to be an uncoupler of photophosphorylation, whereas inhibition of photosynthetic electron transport occurred at higher concentrations. The site of this inhibition was before photosystem II. The uncoupling effect was partially reversible. In a series of related compounds the uncoupling activity appeared mainly dependent on the lipophilic properties of the compounds but electronic effects also played a distinct role. Comparing these uncoupling data with those of some known series of uncouplers it appeared that lipophilicity determines the uncoupling activity in an identical way and a regression equation was found describing the uncoupling activities of 60 compounds belonging to four chemically different groups of compounds.

Introduction

Since the first paper by Krogmann *et al.* [1] on the uncoupling of photophosphorylation by ammonium salts, the effects of many amines on ATP formation in chloroplasts have been described [2, 3]. The effects appeared to be pH-dependent and the uncharged form was thought to be the active species [2, 3]. Ammonium chloride and methylamine are rather weak uncouplers but the uncoupling activity increased considerably on lengthening of the alkyl chain [4]. Some lipophilic chemicals carrying a dialkylaminoalkyl side chain such as chlorpromazine [5, 6], atebrin [6, 7], chloroquine [8] and the series of Wright *et al.* [9] also proved effective uncouplers. A few more complicated lipophilic nitrogenous bases such as octylguanidine [6, 10] and arylaminotetrahydropyrimidines [11] proved to be inhibitory uncouplers.

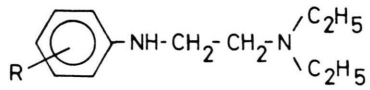
In this paper we wish to report on the oncoupling properties of a series of chemicals related to N,N-diethyl-N′-(4-cyclohexylphenyl)-1,2-ethanediamine (PH 40-62) which also showed algicidal activity against filamentous algae in field conditions [12].

Abbreviations: DAD, diaminodurene; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; FeCy, ferricyanide; MV, methylviologen; PD, *p*-phenylenediamine; PMS, phenazine methosulfate; TMPD, N,N,N′,N′-tetramethyl-*p*-phenylenediamine.

* Reprint requests to: G. van den Berg.

0341-0382/84/0100-0107 $ 01.30/0

A comparison with other uncouplers was made.



PH 40-62 (R = 4-cyclohexyl)

2. Methods

Chemicals

All inhibitors were synthesized in the Synthesis Department of the Duphar Crop Protection Division, by known methods.

Photosynthetic measurements

The isolation of spinach chloroplasts, their heat treatment or digestion by trypsin, and the incubation procedure for measuring photosynthesis have been described recently [11]. Inhibitors were added to the incubation medium as methanolic solutions, the final methanol concentration being always 2%.

Structure-activity relationships

The relationship between chemical structure and biological activity was investigated using the Hansch approach [13]. At first, the hydrophobic and electronic parameters π and σ were used. In order to compare chemically different series it is necessary to use log P values (partition coefficient in an octanol/water system). Sometimes these values were

listed in Tables of Hansch and Leo [13] or given in the original literature. If not, log *P* values were calculated using fragment constants as described by Rekker [14].

3. Results and Discussion

Effects on electron transport and photophosphorylation

Figure 1 shows the effects of different concentrations of PH 40-62 on photoreactions by chloroplasts. At rather low concentrations, 10 μM, the ATP formation is already inhibited up to 50%, while no effects on the electron transport are observed. What happens at higher concentrations depends on the pH of the incubation medium. At pH 7.5 a stimulation of phosphorylating and basal electron transport to 122 and 193% respectively at 0.3 mM occurs. This is a typical picture of an uncoupler. By contrast, at pH 8.0 an inhibition of the electron transport above 0.1 mM is obvious, so that the stimulation of basal and phosphorylating electron transport is eliminated. The fact that uncouplers act as electron transport inhibitors at high pH values has already been known for a long time [2, 3] but in those cases the uncoupling was also pH-dependent, while uncoupling by PH 40-62 is not. The pI_{50} values (negative logarithm of the concentration producing 50% inhibition) can be determined from the dose/response curves. Table I shows these pI_{50} values of PH 40-62 for several electron transport pathways. The different types of phosphorylation are inhibited for 50% at about 8 μM. The effects of 0.5 mM PH 40-62 on different types of photoreductions were studied to establish the inhibition site more precisely; see Table II. The inhibition of FeCy reduction cannot be reversed by PD and there is no effect on photosystem I alone (measured as TMPD/asc → MV). Besides normal chloroplasts, trypsin-treated chloroplasts and heated chloroplasts lacking the water-splitting enzyme were used. In heat-treated chloroplasts the electron transport could be started by ascorbic acid. This electron transport was sensitive to DCMU but not to PH 40-62. The electron transport from TMPD/asc to MV and from asc to MV (heated chloroplasts) was not inhibited by PH 40-62 but showed stimulation up to 190% indicating that uncoupling occurs under these conditions. The time course of trypsin treatment and the dose/response curve with trypsin-treated chloroplasts are presented in Figure 2. Whereas the inhibition of electron transport by DCMU is reversed by trypsin treatment, the inhibition by PH 40-62 is not. Moreover, 0.1 mM of PH 40-62 inhibited the electron transport in trypsin-treated chloroplasts considerably, while the same concentration produced a small stimulation of electron transport in untreated chloroplasts. The stimulation

Table I. pI_{50} values of PH 40-62.

Electron transport pathway (ADP and P_i present)	pI_{50} Photophosphorylation	pI_{50} Electron transport
$H_2O \rightarrow FeCy$	5.02	3.74
$H_2O \rightarrow MV$	5.30	3.87
DAD/asc → MV (10 μM DCMU)	5.30	–
PMS (N_2)	4.99	–

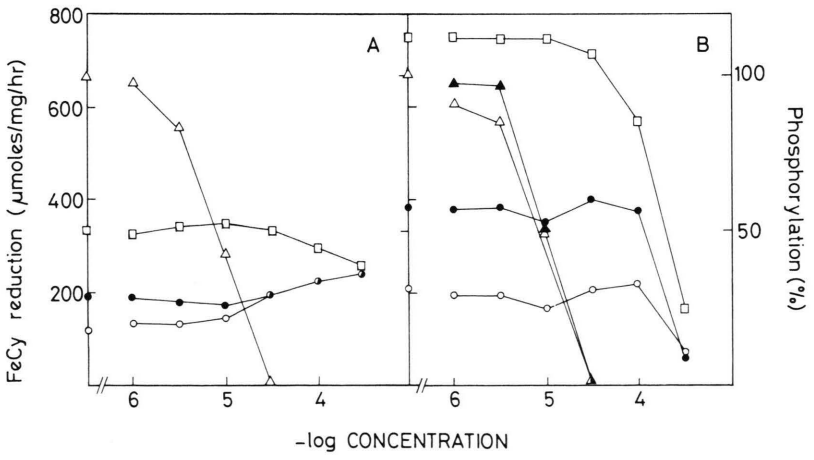


Fig. 1. Hill reaction and photophosphorylation as a function of the concentration of PH 40-62; activities are measured in a HEPES buffer, pH 7.5 (A) or Tricine buffer, pH 8.0 (B). ○ basal electron transport, ● phosphorylating electron transport, △ corresponding phosphorylation, □ uncoupled electron transport (2 mM NH_4Cl) and ▲ cyclic phoshorylation (PMS under N_2).

Table II. Effects of PH 40-62 on different photoreductions in isolated spinach chloroplasts.

Pathway	Basal electron transport, pH 8.0		
	Control rate [μmol/mg/h][a]	Activity in % of control	
		1 μM DCMU	0.5 mM PH 40-62
Control chloroplasts			
$H_2O \rightarrow$ FeCy	210	6	7
$H_2O \rightarrow$ FeCy (0.1 mM PD)	363	0	5
$H_2O \rightarrow$ MV	59	0	30
TMPD/asc → MV (10 μM DCMU)	180	–	190
Trypsin treated chloroplasts[b]			
$H_2O \rightarrow$ FeCy	220	44	0
Heated chloroplasts			
asc → MV	60	0	157

[a] μmol FeCy reduced or μmol O_2 consumed.
[b] Pre-treated with trypsin for 2 min.

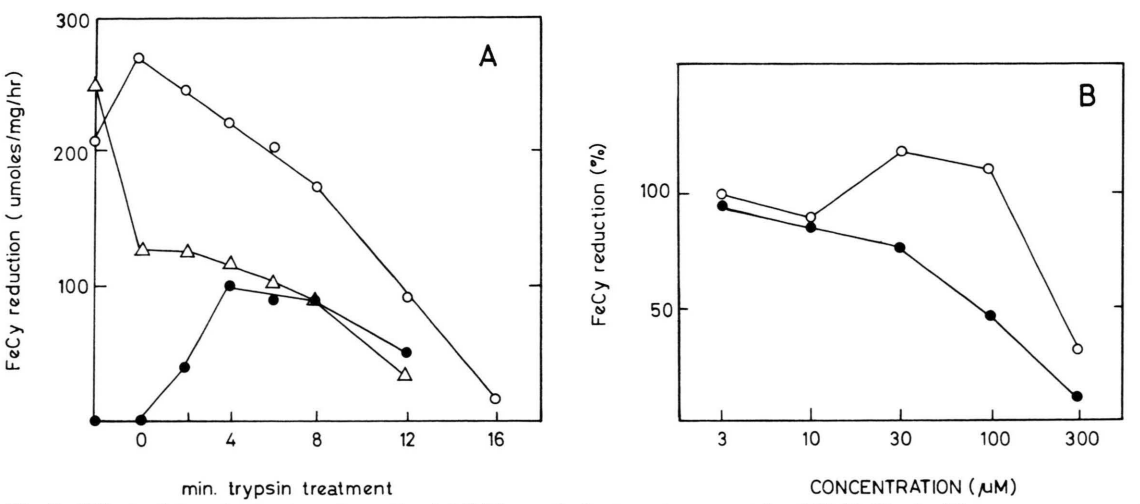


Fig. 2. Effect of trypsin treatment on the inhibition of electron transport by PH 40-62. A. Time course experiment, ○ control, ● 1 μM DCMU, △ 0.1 mM PH 40-62. B. Dose/response curve, ○ control chloroplasts, ● chloroplasts treated with trypsin for 2 min.

of the electron transport due to uncoupling by PH 40-62 has disappeared totally upon trypsin treatment (Figure 2B). These experiments indicate that PH 40-62 inhibits the electron transport before photosystem II near the water-splitting site, as has been described for other types of amine uncouplers [11, 15] but uncouplers of other types also inhibit at the water-splitting site [16, 17].

Reversibility of the inhibition

To investigate whether or not the effects of PH 40-62 are reversible, chloroplasts were pre-incubated in a buffer (50 mM Tricine pH 8.0, 1 mM $MgCl_2$) containing different amounts of this compound. After 10 min incubation in the dark the inhibitor was removed by washing and centrifugation at 30 000 × *g*. Photosynthetic activities were measured in the standard medium. The results, shown in Table III, indicate irreversible inhibition of the elecetron transport at 1 mM. Uncoupling by PH 40-62 is partially reversible while effects of NH_4Cl are fully reversible. The latter is in agreement with other studies [2, 3]. Avron and Shavit studied the nature of the effects by chlorpromazine

Table III. Reversibility of the effects of PH 40-62.

Inhibitor	Concentration [mM]	FeCy reduction [μmol/mg/h]	ATP/2*e* ratio
Control		286 (210)	0.99 (0.82)
NH_4Cl	1	369 (233)	0.39 (0.79)
	10	274 (258)	0.11 (0.87)
	100	74 (256)	0.00 (0.65)
PH 40-62	0.01	226 (225)	0.45 (0.75)
	0.1	215 (214)	0.00 (0.57)
	1	20 (15)	0.00 (0.29)

Activities after removal of the uncoupler by washing in brackets. Means of two experiments.

and atebrin [6], and also found partially reversible uncoupling by these chemicals. Other uncouplers such as methylamine [2, 3] and carbonylcyanide phenylhydrazones [18] uncouple in a reversible way.

Structure-activity relationships

In 1960 Good showed that simple alkylamines were uncouplers while more polar compounds like 1,2-diaminoethane were not [2]. Later on, Crofts showed that the tetra-N-alkylated derivative did show uncoupling properties [19]. Other chemicals having dialkylaminoalkyl side chains such as chlorpromazine, atebrin and PH 40-62 proved uncouplers as well [5–9]. In order to ascertain which portion of the molecule is essential to the uncoupling activity we studied the effects of a series of derivatives of PH 40-62 in which the substituents in the phenyl ring are changed. Table IV shows the activities of these chemicals on FeCy reduction and phosphorylation.

Only the most lipophilic compounds including PH 40-62 inhibit FeCy reduction below 0.3 mM. The effects on cyclic and noncyclic phosphorylation are almost identical. The pI_{50} values increase with increasing length of the alkyl chain indicating the possible role of hydrophobic binding. The uncoupling activities were analysed using multiple regression and a good correlation with π and σ was found, whereas π showed a quadratic relationship. For the noncyclic and cyclic phosphorylation Eqs. (1) and (2) were found respectively.

$$pI_{50} = 3.825 + 0.592\,\pi - 0.066\,\pi^2 + 0.353\,\sigma \quad (1)$$
$$(7.190)\ (-3.163)\ (3.927)$$
$$n = 19\,,\quad r = 0.942\,,\quad s = 0.145\,,\quad F = 45.09$$

$$pI_{50} = 3.832 + 0.587\,\pi - 0.070\,\pi^2 + 0.523\,\sigma \quad (2)$$
$$(6.968)\ (-3.268)\ (5.687)$$
$$n = 19\,,\quad r = 0.936\,,\quad s = 0.148\,,\quad F = 40.21\,.$$

In the above presentation n is the number of compounds, r is the correlation coefficient, s is the standard error of the estimate and F represents the overall statistical significance of the equation.

Lipophilicity appears to play a major role and optimum activity is reached when π is 4.5 and 4.2 respectively, which corresponds to an octyl substituent. However, because compounds having more lipophilic substituents are lacking in this series there is no proof of a real optimum. The σ terms suggest that electron-withdrawing groups will increase the uncoupling activity. The quality of Eqs. (1) and (2) is quite good: the differences between the observed and the calculated values in Table IV are rather small, no matter whether the substituents are introduced into ortho, meta or para position.

Changes at the other end of the molecule have only a slight effect on the uncoupling activity; see Table V. A similar conclusion was drawn by Wright *et al.* for their series of uncouplers [9]. Only methyl substitution at the aniline nitrogen caused an increase in uncoupling activity (see compound **22** in Table V).

Comparison between the PH 40-62 series and other series of uncouplers

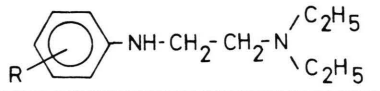
The chemically simplest uncouplers are *n*-alkylamines and McCarty and Coleman found their activity to be linear correlated with log *P* [4]. Our QSAR analysis led to Eq. (3).

$$pI_{50} = 2.288 + 0.472 \log P \quad (3)$$
$$(11.578)$$
$$n = 8\,,\quad r = 0.978\,,\quad s = 0.139\,,\quad F = 134.05\,.$$

In studying alkylamines with longer alkyl chains, Trebst *et al.* found that optimum uncoupling activity is reached with dodecylamine, which has a log *P* value of about 5.1 [20].

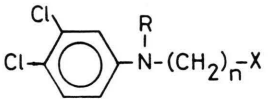
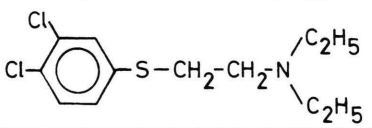
We calculated the log *P* value of compound **1**, according to the method of Rekker [14] and found a value of 2.31. A regression analysis using the uncoupling of cyclic phosphorylation and calculated log *P* values of the compounds listed in Tables IV

Table IV. Effects of N,N-diethyl-N′-arylethanediamines on photophosphorylation and electron transport in spinach chloroplasts.

Nr.	Structure	pI_{50} [M^{-1}]				
		Electron transport	Noncyclic phoshorylation		Cyclic phosphorylation	
			obsvd.	calcd. [a]	obsvd.	calcd. [b]
1	R = H	< 3.5	3.80	3.82	3.90	3.83
2	4-CH_3	< 3.5	4.00	4.05	3.96	4.03
3	2-*i*-C_3H_7	< 3.5	4.40	4.53	4.48	4.50
4	4-*n*-C_4H_9	< 3.5	4.98	4.71	4.82	4.66
5	4-*t*-C_4H_9	< 3.5	4.74	4.70	4.75	4.64
6	4-*n*-C_6H_{13}	4.09	4.98	4.97	5.02	4.90
7 (PH 40-62)	4-*c*-C_6H_{11}	3.74	5.02	4.88	4.99	4.80
8	4-*n*-C_8H_{17}	4.27	4.98	5.08	4.80	4.98
9	2,4-$(CH_3)_2$	< 3.5	4.27	4.25	4.01	4.19
10	4-OCH_3	< 3.5	3.72	3.78	3.71	3.74
11	3-CF_3	< 3.5	4.34	4.57	4.57	4.64
12	4-CF_3	< 3.5	4.45	4.61	4.63	4.70
13	2-Cl	< 3.5	4.23	4.31	4.20	4.35
14	3-Cl	< 3.5	4.31	4.36	4.29	4.42
15	4-Cl	< 3.5	4.41	4.31	4.44	4.35
16	2,4-Cl_2	< 3.5	4.80	4.72	4.86	4.79
17	3,4-Cl_2	< 3.5	4.70	4.77	4.70	4.86
18	4-NO_2	< 3.5	4.14	3.95	4.31	4.09
19	4-SO_2CF_3	< 3.5	4.55	4.46	4.64	4.62

[a] Calculated using Eq. (1).
[b] Calculated using Eq. (2).

Table V. Effects of some PH 40-62 analogs on photophosphorylation in spinach chloroplasts.

Nr.	Structure	pI_{50} [M^{-1}]	
		Noncyclic phosphorylation	Cyclic phosphorylation
17	R = H n = 2 X = $N(C_2H_5)_2$	4.70	4.70
20	H 2 $NH(i\text{-}C_4H_9)$	4.75	4.69
21	H 3 $N(C_2H_5)_2$	4.78	4.74
22	CH_3 2 $N(C_2H_5)_2$	4.87	4.94
23		4.77	4.76

and V yielded:

$$pI_{50} = 2.363 + 0.891 \log P - 0.077 \log P^2 \quad (4)$$
$$(3.472) \qquad (-2.486)$$
$$n = 23\,, \quad r = 0.814\,, \quad s = 0.225\,, \quad F = 21.12\,.$$

The quality of this equation is much less than that of Eq. (2) because the influence of electronic effects is ignored. The optimum $\log P$ value is 5.8. We applied the same procedure to the chemicals of our previous paper [11] and found the $\log P$ value of 2-phenylamino-1,4,5,6-tetrahydropyrimidine to be 1.0. For a series of 12 derivatives Eq. (5) was found:

$$pI_{50} = 2.183 + 1.259 \log P - 0.135 \log P^2 \quad (5)$$
$$(9.473) \qquad (-8.038)$$
$$n = 12\,, \quad r = 0.965\,, \quad s = 0.126\,, \quad F = 66.54\,.$$

The optimum $\log P$ value is 4.7. It is noteworthy that in the three series discussed so far the maxi-

mum pI_{50} value for uncoupling is always about 5.0, while the optimum log P value is also about 5.0. The uncoupling data of the three series are plotted against log P in Fig. 3 and for the whole group of uncouplers an equation of surprisingly good quality was obtained:

$$pI_{50} = 2.106 + 1.004 \log P - 0.087 \log P^2 \quad (6)$$
$$(8.533) \qquad (-5.093)$$
$$n = 43\,, \quad r = 0.901\,, \quad s = 0.332\,, \quad F = 89.02\,.$$

This equation is illustrated in Fig. 3. The aliphatic amines (open circles) are less active than predicted. This discrepancy can be caused by differences in experimental procedures. Another explanation is that aromatic compounds are more active uncouplers than aliphatic compounds having the same lipophilicity, because of electronic effects. Because the chemical structures of the compounds mentioned in Fig. 3 are rather different it is not possible to use a σ parameter for these 43 compounds in a simple way. We therefore decided to add a dummy parameter which has the value of one for aliphatic amines and is equal to zero for the aromatic compounds. Now Eq. (7) was obtained.

$$pI_{50} = 2.887 + 0.694 \log P - 0.057 \log P^2 - 0.730\, D$$
$$(6.799) \qquad (-4.179) \qquad (-5.830) \quad (7)$$
$$n = 43\,, \quad r = 0.947\,, \quad s = 0.249\,, \quad F = 119.63\,.$$

Two complicated amines reported in the literature to be uncouplers *viz.* chlorpromazine [5, 6] and atebrin [6, 7] can be added to the series without changing the correlation significantly (these compounds are indicated in Fig. 3 by asterisks).

The phenomena discussed so far are apparently typical of uncouplers of the amine type. In contrast, a second type of inhibitors of ATP formation has been described having an aromatic $-NH-$ group which has acidic rather than basic properties.

Examples are benzimidazoles [20, 21], thiadiazolylanilides [22, 23], diphenylamines [17, 24] and phenylhydrazones [6]. In these instances, the introduction of a methyl group at the acidic $-NH-$ totally eliminated the encoupling activity [21, 23], whereas the nitrogen atoms of the basic uncouplers may be completely substituted (see for example, compound **22**). Moreover, in the PH 40-62 series the most basic nitrogen atom is not the anilide nitrogen but the diethylaminoethylene-group having a pK_a of about 9.4 which is comparable to that of NH_4^+. The second type of uncouplers mostly inhibit the electron transport in such a way that only the effects on cyclic phosphorylation can be measured. In consequence, the question whether these compounds are inhibitory uncouplers of inhibitors of energy transduction is not well documented. Structure activity relationship studies with these compounds showed electronic effects to be most important, since the uncoupling activity increases with the number of electronegative substituents [23, 24]. The most active compounds in the series of Schäfer *et al.* [23] and Oettmeier [24] have pI_{50} values of 7.30 and 6.74 and corresponding log P values of 3.0 and 3.7. Moreover, Renger described an anilinothiophene having a pI_{50} of even 7.7 and a calculated log P of

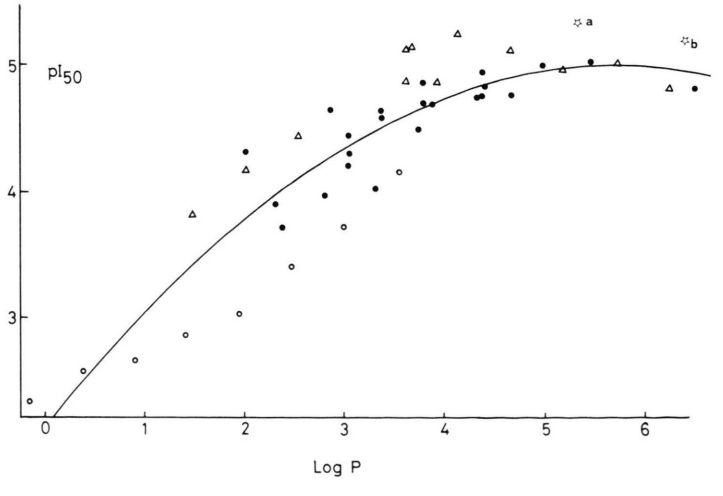


Fig. 3. Relationship between uncoupling of cyclic photophosphorylation (pI_{50}) and partition coefficient in octanol/water (log P). ○ aliphatic amines (from ref. 4), △ 2-arylamino-1,4,5,6-tetrahydropyrimidines (from ref. 11), ● alkylated N-aryl-1,2-ethanediamines (from Tables IV and V), a, chlorpromazine, b, atebrin.

4.7 [16, 25]. These compounds are polysubstituted by halogen and nitro groups producing an electron-deficient −NH− group and they do not fit easily into the picture of Fig. 3. On the contrary, for the series of thiadiazolylanilides [23] we found a correlation with pK_a:

$$pI_{50} = 9.785 - 0.599\,pK_a \qquad (8)$$
$$(-6.185)$$
$$n = 14\,,\quad r = 0.872\,,\quad s = 0.528\,,\quad F = 38.25\,.$$

and Oettmeier described a relation with σ for diphenylamines [24]. In another thiadiazolylanilide series a relation with log P was found [22], but this relation is not very reliable due to the small number of compounds and a relationship between uncoupling and electronic effects cannot be excluded. However, for the series of benzimidazoles an excellent correlation with log P was found by Büchel *et al.* [21]. These log P values are however, not related to Hansch's π values ($r = 0.43$) and the uncoupling activity showed very poor correlation with π, σ and pK_a values.

It is surprising to note that 15 benzimidazoles from the paper by Büchel *et al.* [21] can be added to the 45 compounds presented in Fig. 3 by using the experimentally determined log P values without producing any substantial deterioration in the correlation:

$$pI_{50} = 1.949 + 0.991\log P - 0.076\log P^2 \qquad (9)$$
$$(8.192)\qquad(-4.457)$$
$$n = 60\,,\quad r = 0.882\,,\quad s = 0.373\,,\quad F = 101.57\,.$$

This equation should be compared directly with Eq. (6).

Only the unsubstituted 2-CF_3-benzimidazole has been omitted because the difference between the determined and predicted pI_{50} value was more than 1.0.

Summarizing, we can conclude that there are two types of uncouplers:

1. the type of basic amino derivatives for which lipophilicity is the most important property and electronic effects play only a secondary but obvious role (see Eqs. (1) and (2)).
2. the compounds having an −NH− group between two aromatic systems in which electronic effects are much more important than lipophilicity and of which the −NH− hydrogen must not be alkylated. These compounds are generally lipophilic weak acids, which property fits well into the chemiosmotic theory for uncoupling. In a few instances, however, a correlation with experimentally determined log P was found although these log P values were not related to Hansch's π constants.

The real uncoupling mechanism of both types is not clear but it is probably more complicated than that of simple compounds such as methylamine and ammonium chloride.

Acknowledgements

The authors are indebted to Dr. G. B. Paerels and his colleagues who synthesized the compounds listed in Tables IV and V and to Mr. J. Tipker for assistance with the computer calculations.

[1] D. W. Krogmann, A. T. Jagendorf, and M. Avron, Plant Physiol. **34,** 272–277 (1959).
[2] N. E. Good, Biochim. Biophys. Acta **40,** 502–517 (1960).
[3] G. Hind and C. P. Whittingham, Biochim. Biophys. Acta **75,** 194–202 (1963).
[4] R. E. McCarty and C. H. Coleman, Arch. Biochem. Biophys. **141,** 198–206 (1970).
[5] J. S. C. Wessels and H. Baltscheffsky, Acta Chem. Scand. **14,** 233–246 (1960).
[6] M. Avron and N. Shavit, Biochim. Biophys. Acta **109,** 317–331 (1965).
[7] H. Baltscheffsky, Acta Chem. Scand. **14,** 264–272 (1960).
[8] G. Hind, H. Y. Nakatani, and S. Izawa, Biochim. Biophys. Acta **172,** 277–289 (1969).
[9] B. J. Wright, A. C. Baillie, K. Wright, J. R. Dowsett, and T. M. Sharpe, Phytochemistry **19,** 61–65 (1980).
[10] J. Mottley, Pest. Biochem. Physiol. **9,** 340–350 (1978).
[11] G. van den Berg, M. Brandse, and J. Tipker, Z. Naturforsch. **37 C,** 651–657 (1982).
[12] D. H. Spencer-Jones and C. W. Raven, Proc. Symp. Aquatic Weeds and their control, Oxford 7–8 April 1981, Ass. Appl. Biol. 177–182.
[13] C. Hansch and A. J. Leo, Substituent Constants for Correlation Analysis in Chemistry and Biology, J. Wiley, New York 1979.
[14] R. F. Rekker, The hydrophobic fragmental constant. Its derivation and application. A means of charcaterizing membrane systems, Elsevier, Amsterdam 1977.
[15] S. Izawa, R. L. Heath, and G. Hind, Biochim. Biophys. Acta **180,** 388–398 (1969).

[16] G. Renger, B. Bouges-Bocquet, and K. H. Büchel, Bioenerg. **4,** 491–505 (1973).
[17] W. Oettmeier and G. Renger, Biochim. Biophys. Acta **593,** 113–124 (1980).
[18] D. Y. de Kiewiet, D. O. Hall, and E. L. Jenner, Biochim. Biophys. Acta **109,** 284–292 (1965).
[19] A. R. Crofts, Biochem. J. **105,** 29P (1967).
[20] A. Trebst, E. Pistorius, and K. H. Büchel, Z. Naturforsch. **21 B,** 667–672 (1966).
[21] K. H. Büchel, W. Draber, A. Trebst, and E. Pistorius, Z. Naturforsch. **21 B,** 243–254 (1966).
[22] P. Bracha, M. Luwisch, and N. Shavit, Proc. 2nd IUPAC Congress on Pesticide Chem. V (Ed. A. S. Tahori), 141–151, Gordon & Breach Sci. Publ. New York 1972.
[23] G. Schäfer, A. Trebst, and K. H. Büchel, Z. Naturforsch. **30 C,** 183–189 (1975).
[24] W. Oettmeier, Z. Naturforsch. **34 C,** 1024–1027 (1979).
[25] G. Renger, FEBS Letters **23,** 321–324 (1972).