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SYNTHESIS OF POTENTIAL HERBICIDES DESIGNED TO UNCOUPLE PHOTOPHOSPHORYLATION

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Abstract—In a search for novel herbicides we attempted to make uncouplers of photophosphorylation. Good herbicides were discovered, but not all were good uncouplers and we present evidence which supports the view that their primary action is through inhibition of carotenoid biosynthesis. This paper describes the synthesis and *in vivo* and *in vitro* activities of 21 compounds of the series. The best herbicide was active enough to justify extensive field testing.

INTRODUCTION

For some years we have been interested in using biochemical reasoning to generate leads for the discovery of new pesticides [1]. For instance we have made inhibitors of indole acetic acid oxidase in an attempt to simulate the effect of the auxin herbicides [2], and inhibitors of choline acetyltransferase as potential insecticides [3].

As part of this programme we have now prepared a variety of strongly basic lipophilic amines as potential herbicides. Compounds of this type are known to be good uncouplers of photophosphorylation *in vitro* [4] since they can penetrate the thylakoid membrane and dissipate the H^+ gradient which, according to the chemiosmotic theory, drives ATP synthesis [5]. Photophosphorylation is thus a primary source of chloroplast ATP in plants and we therefore hoped that these compounds might be herbicidal. With this aim in view we have investigated a series of aryloxyalkylamines of general structure A, $ArO(CH_2)_nNR_2$ where Ar represents an aromatic moiety, R is an alkyl group (R_2 can also be a ring system) and $n = 2$, as potential uncouplers and herbicides. Only a few simple compounds of structure A (Ar = substituted phenyl) were already patented as herbicides [6, 7] but their uncoupling activity has not been investigated.

During the course of our programme Yokoyama and his colleagues showed that 2-(4-chlorophenylthio)-triethylamine HCl (CPTA) and related materials were inhibitors of carotenoid biosynthesis *in vivo* (see [8] and *loc. cit.*) and accordingly we conducted experiments to investigate whether our compounds behaved in the same way.

Many of our materials were herbicidal and the most active of these underwent field trials for several years. It was both an uncoupler of photophosphorylation and an inhibitor of carotenoid biosynthesis in the systems examined. This paper describes the synthesis and both the *in vivo* and *in vitro* activities of this and other selected compounds of the series.

RESULTS

In vitro *uncoupling activity*

Preliminary experiments were carried out to determine the structural criteria required for maximum *in vitro* activity with the following conclusions.

(i) Only small variations in uncoupling activity were observed when we altered the alkylaminoalkyl side-chain (Table 1). Increasing the number of methylene groups between the oxygen and nitrogen from 2 to 3 made the uncouplers marginally more effective. Activity was virtually independent of the alkyl groups attached to the nitrogen atom but in the case of ring systems the morpholino compound **8** was much less

Table 1. Effect of variations in the alkylamine side-chain on the *in vitro* uncoupling activities of compounds of structure *p*-(*t*Bu)—C_6H_4—$O(CH_2)_nNR_2$

Compound	$R(R_2)$	n	50% uncoupling concn (μM)
1	Me	3	26
2	Me	2	42
3	Et	3	36
4	Et	2	49
5	*n*Pr	2	49
6	*n*Bu	2	46
7		2	38
8		2	3% at 100 μM

Uncoupling activity was measured in a standard assay system described in the Experimental.

Table 2. Effect of lipophilicity on the *in vitro* uncoupling activities of compounds of structure $ArOCH_2CH_2NEt_2$

Compound	Ar	Uncoupling activity
9	Ph	21% at 100 μM
10	*p*-Me—C_6H_4	27% at 100 μM
11	α-$C_{10}H_7$	50% at 51 μM
4	*p*-(*t*Bu)—C_6H_4	50% at 49 μM
12	*p*-Ph—C_6H_4	50% at 45 μM
13	Ph_3C	50% at 14 μM

Uncoupling was measured in a standard assay system described in the Experimental.

active than the piperidino analogue **7**, presumably because morpholine is a weaker base than piperidine. Due to its ready availability the diethylaminoethyl group was invariably used in subsequent synthesis.

(ii) In agreement with previous work [9], an increase in lipophilicity caused an increase in uncoupling activity (Table 2). Only selected compounds have been included to exemplify the general trend. Aliphatic substituents in the aromatic nucleus, which cause an increase in lipophilicity, also enhanced uncoupling activity.

Many of our compounds were good uncouplers *in vitro* (50% uncoupling concentrations in the range 10–50 μM). This led us to hope that if this activity were expressed in the whole plant then a herbicidal effect would be observed (see later).

In vivo *activity*

As we had hoped, many of our early compounds were indeed herbicidal. They caused pronounced chlorosis, and were most active when applied post-emergence to dicotyledonous plants (Table 3).

Table 3 also indicates the enhanced activity obtained with *bis*-aryl materials (i.e. **12** and **15**). This led us to concentrate further efforts on compounds of the general structure B, *p*-(PhX)—$C_6H_4OCH_2CH_2NEt_2$, where X represents a linking group.

We synthesized and tested as herbicides a wide range of such compounds. Table 4 shows the activity of these on a range of dicotyledonous weeds in more detailed tests. The acetylene **21** was the most active.

The effects of a number of other modifications to molecules of structure B were examined, and we found that activity was diminished when we made the following changes:

(i) substitution of the aromatic nucleus (primarily in the terminal ring for synthetic reasons);
(ii) attachment of the diethylaminoethoxy group to the *meta*- and *ortho*-positions relative to the linking group X; and
(iii) replacement of the oxygen atom in the side-chain by sulphur, carbonyl or an NH group.

Inhibition of carotenoid biosynthesis

As mentioned earlier, CPTA has been shown to inhibit β-carotene biosynthesis *in vivo* [8] and cause an accumulation of lycopene. This suggests that it inhibits the cyclization steps in the final stages of carotenoid biosynthesis. Since β-carotene is required for stabilization of chlorophyll in the primary photosynthetic unit [10], inhibition of carotenoid biosynthesis would be expected to cause plant death, and is in fact thought to be the primary biochemical effect of, for example, norflurazon [11] and aminotriazole [12].

We tested a range of our compounds as inhibitors of carotenoid formation in dark-grown pumpkin cotyledons which were placed in the light at the commencement of treatment with compound. Our results were similar to those reported for CPTA [13]. Visually there was a reduction in the amount of chlorophyll produced and the treated cotyledons developed a pinkish colouration in the chlorotic areas, possibly due to lycopene accumulation.

In an experiment with compound **17**, cotyledons treated with different concentrations for a fixed time

Table 3. *In vivo* activity of compounds of structure $ArOCH_2CH_2NEt_2$

Compound	Ar	Application rate (kg/ha)	Post-emergence activity P	M	L	S	R	O	Total score*
9	Ph	11.2	3	3	5	6	1	2	20
4	*p*-(*t*Bu)—C_6H_4	2.8	3	6	7	3	1	1	21
12	*p*-Ph—C_6H_4	2.8	5	7	7	8	4	5	36
14	β-$C_{10}H_7$	2.8	2	1	2	2	1	1	9
15	*p*-PhO—C_6H_4	2.8	4	7	5	7	2	4	29

Plants (14 days old) were sprayed with chemical in an aq. soln, aq. suspension or aq. Me_2CO solution according to solubility, to give an application rate equivalent to that stated. The post-emergence herbicidal activity was visually assessed 14 days later and expressed on a scale 0–9 as follows:

Percentage damage	0	0–10	10–24	25–34	35–49	50–64	65–74	75–84	85–94	95–100
Score	0	1	2	3	4	5	6	7	8	9

* The total score is the sum of the individual scores.

The following species were used: pea (P)—*Pisum sativum* cv Onward; mustard (M)—*Sinapis alba*; linseed (L)—*Linum usitatissimum*; sugarbeet (S)—*Beta vulgaris* cv Sharpe's Klein E; ryegrass (R)—*Lolium perenne*; oats (O)—*Avena sativa* cv Condor.

Table 4. *In vivo* activity against important weed species of compounds of structure p-(Ph—X)—$C_6H_4OCH_2CH_2NEt_2$ (**B**)

Compound	X	Post-emergence activity (0.7 kg/ha) Ch	My	Cl	Pp	F	Pg	Total score
12	—	7	4	6	2	5	8	32
15	—O—	2	4	4	2	3	3	18
16	—N=N—	8	4	7	0	6	5	30
17	—CH_2O—	8	5	7	2	6	6	34
18	—NH·NH—	8	6	8	2	6	4	34
19	—CH_2·CH_2—	9	7	8	1	4	5	34
20	—CH=CH—	8	7	8	1	5	8	37
21	—C≡C—	9	8	8	1	5	8	39

The means of application of chemical and the basis for assessment of activity are described in the legend to Table 3. Weed species; chickweed (Ch)—*Stellaria media*; mayweed (My)—*Tripleurospermum maritimum*; cleavers (Cl)—*Galium aparine*; pale persicaria (Pp)—*Polygonum lapathifolium*; fat hen (F)—*Chenopodium album*; pigweed (Pg)—*Amaranthus retroflexus*.

were homogenized and the chlorophyll and carotenoids separated and quantified. As the dose of compound was increased more lycopene accumulated and the chlorophyll content fell in parallel with that of β-carotene (Fig. 1).

This latter finding allowed us to test the effect of a number of compounds on the pigment content of treated cotyledons routinely since it was much quicker and easier to assay the chlorophyll content as an indication of the inhibition of β-carotene biosynthesis.

Table 5 shows the effect of a number of selected compounds on chlorophyll biosynthesis (and by inference on carotenoid biosynthesis) and, for comparison, also gives their *in vitro* uncoupling activity and herbicidal properties.

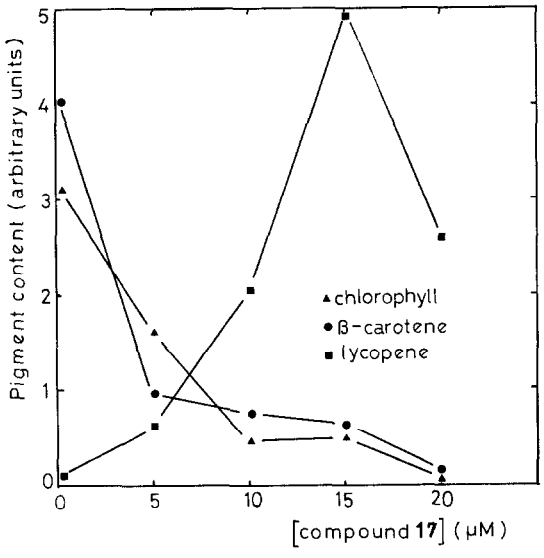

Fig. 1 The effect of compound **17** on lycopene, β-carotene and chlorophyll content of pumpkin cotyledons. A fixed proportion of each carotenoid extract was separated as described, and the carotenoid content measured from the *A* at the absorption maximum. It was then divided by the growth index (Experimental) to allow for different extents of growth of the cotyledons. The units used in the figure are arbitrary, chosen to show the pattern of the changing amounts.

Symptoms of treated plants kept in the light and dark

French bean plants at the 2 leaf stage were sprayed with compounds **15**, **20** and aminotriazole (at the rate of 0.7 kg/ha) with appropriate controls. Plants were kept either in light or darkness for one week.

All compounds caused chlorosis in the light, and the aryloxyalkylamines also caused scorching. In the dark, however, leaves treated with compounds **15** and **20** were withered but still green. Shoots were etiolated, as in the control, but their growth was retarded. A pink tinge to the new shoots was particularly evident in the plants treated with compound **20** in the dark. The overall height of the plants was not greatly affected in the light (100 mm high when sprayed, 120–130 mm high after 1 week) but in the dark control plants reached *ca* 160 mm whilst treated plants were 120–130 mm tall.

Concentration of compound **15** *in treated plants*

Pea plants *ca* 100 mm tall were sprayed with compound **15** at a rate of 0.7 kg/ha, and after various times the green parts of the plants were washed to remove surface material, extracted, and the amount of compound present measured by GLC. On the approximation that 1 g plant material contains 1 ml H_2O and the assumption that the compound can cross biological membranes so that it becomes uniformly distributed through this volume, the average amount inside the plants rose to the equivalent of *ca* 40 μM after 24 hr.

DISCUSSION

In our search for novel herbicides we set out to make potent uncouplers of photophosphorylation and this aim was achieved. Furthermore, some of the uncouplers were good herbicides. For instance the best herbicide, **21**, caused 34% uncoupling at 20 μM. This compound had to be applied at *ca* 2 kg/ha for good herbicide activity. Thus, assuming that **21** penetrated as well as **15** whose uptake was estimated (a level equivalent to 40 μM at 0.7 kg/ha after 24 hr), and that it was uniformly distributed throughout the green plant tissue, then there might have been the equivalent of *ca* 100 μM **21** within the cells and severe uncoupling could have resulted and contributed to the herbicidal effect.

Table 5. Comparison of *in vitro* and *in vivo* activities of herbicidal alkylamines

Compound No.	Structure	Uncoupling activity	Chlorophyll accumulation inhibition at 10 μM (%)	Post-emergence herbicide data (2.8 kg/ha) P	M	L	S	R	O	Total score
15	*p*-(PhO)—$C_6H_4OCH_2CH_2NEt_2$	50% at 32 μM	0	4	7	5	7	2	4	29
17	*p*-($PhCH_2O$)—$C_6H_4OCH_2CH_2NEt_2$	50% at 58 μM	64	5	8	7	8	2	3	33
21	*p*-(Ph—C≡C)—$C_6H_4OCH_2CH_2NEt_2$	34% at 20 μM*	64	5	8	8	8	7	2	38
2	*p*-(*t*Bu)—$C_6H_4OCH_2CH_2NMe_2$	50% at 42 μM	20	3	2	1	2	1	2	11
8	*p*-(*t*Bu)—$C_6H_4OCH_2CH_2$ 	3% at 100 μM	33	3	3	1	4	1	1	13

The means of application of chemical and the basis for assessment of herbicidal activity are described in the legend to Table 3. Uncoupling activity and inhibition of chlorophyll accumulation were measured as described in the Experimental.

* Maximum solubility of compound in assay buffer.

However, this cannot be the whole story since there were modest herbicides which were not uncouplers (e.g. **8**, Table 5). On the other hand, the chlorosis produced by **8** and other compounds is consistent with inhibition of the synthesis of β-carotene which would lead to photodestruction of chlorophyll [10]. In keeping with this interpretation plants kept in the dark after treatment with **15** and **20** did not lose their chlorophyll. However, a theory of the mode of action of our compounds also has to take account of the fact that leaves of plants treated in the dark became desiccated. The chloroplast normally contains the correct proportion of the various compounds of the thylakoid membrane, so it seems likely that a disturbance in the synthesis of one may lead, via normal regulatory processes, to alterations in the synthesis of others. This may be the reason for the wide range of effects observed with, for instance, the pyridazinone class of herbicides whose primary mode of action is probably on carotenoid synthesis [11, 14–16]. Perhaps in our case an over-accumulation of an intermediate such as lycopene might affect the functional integrity of thylakoid and other cellular membranes and bring about desiccation. Certainly this effect is hard to explain solely on the basis of photophosphorylation uncoupling. Naturally it is impossible to rule out effects on processes which we have not investigated so there might even be effects not identified here, but it is reasonable to conclude that the herbicidal effect may be due to a primary influence on carotenoid biosynthesis.

As it happens, neither compounds **15** nor **20** prevented normal greening in detached pumpkin cotyledons, but this was probably a feature of their species specificity. We did not investigate this further but it usually turns out to be due to differences in the extents of uptake and metabolism between plant species [17]. Whilst the evidence suggests that our compounds inhibit carotenoid biosynthesis between lycopene and β-carotene, we do not know whether, in addition, they cause increases in absolute amounts of carotenoids, as does CPTA. Yokoyama and his colleagues [18] hypothesized that the large absolute increases in lycopene that CPTA caused in Marsh seedless grapefruit were due to action at the gene level. However, there does not seem to be any evidence which rules out the simpler suggestion (e.g. [19]) that the removal of the end product relieves the feedback inhibition of the pathway.

In conclusion, the observed effects of the compounds in our *in vitro* systems seem enough to explain their herbicidal activity which in the case of **21** was good enough to warrant extensive greenhouse and field trials although not good enough for the compound to be commercialized. The primary effect is probably on carotenoid synthesis with contributions from uncoupling in some cases. Therefore, although the compounds certainly do not act solely on the biochemical process they were designed to inhibit, the results nevertheless encourage us in the belief that biochemical rationale can be a useful starting point in the search for new pesticides.

EXPERIMENTAL

Synthesis of compounds. Of the compounds exemplified in the tables, all except **2**, **8**, **16**, **18** and **21** have been reported either as the free bases or their HCl salts. All the compounds gave satisfactory elemental analyses and their IR and ^{1}H NMR spectra were in agreement with the proposed structures.

Except for those described below, all the compounds were prepared by the same general method. NaH (50% in oil; 0.1 mol) was added to a stirred soln of the appropriate phenol (0.1 mol) in dry xylene (250 ml) and the mixture was stirred until effervescence had ceased (warming if necessary). A soln of 2-chlorotriethylamine, generated from its HCl salt [20] (0.1 mol) in dry xylene (100 ml) was added dropwise to the stirred refluxing suspension of the Na^+ salt. After 6 hr the mixture was cooled and extracted with H_2O and the xylene layer was dried ($MgSO_4$) and evapd. Fractional distillation of the residue *in vacuo* afforded the required aryloxyalkylamine as a colourless oil. New compound: **16**, bp 166–168° at 0.35 mm Hg.

Synthesis of compounds in Table 1: A soln of *p*-*t*-butylphenol (0.1 mol) in dry DMF (200 ml) was treated at room temp. with NaH (50% in oil) (0.2 mol). After effervescence had subsided the appropriate chloroalkylamine HCl salt (0.1 mol) was added and the resulting soln stirred at 100° for 1 hr.

Dilution with H_2O and extraction with Et_2O, etc., afforded the crude product which was purified by fractional distillation *in vacuo*. New compounds: **2**, bp 197–199° at 12 mm Hg; **8**, bp 140–142° at 0.35 mm Hg.

Compound **13** was prepared by reacting chlorotriphenylmethane with 2-diethylaminoethanol in refluxing xylene followed by treatment with dil NaOH to liberate the free base. Compound **18** was made from **16** by reduction with Zn and HOAc [21] and was obtained as a yellow oil, bp 182–187° at 0.1 mm Hg. Compound **21** was prepared from **20** by the method of ref. [22], bp 172–175° at 0.2 mm Hg.

Measurement of uncoupling activity. Spinach chloroplasts were prepared essentially as described in ref. [23] and chlorophyll assayed by the method of ref. [24]. Photophosphorylation was quantified by a combination of known methods [25, 26] in the presence and absence of compound. The assay system contained, in a final vol. of 3 ml, 150 mM sucrose, 2 mM $MgCl_2$, 3.33 mM $K_3Fe(CN)_6$, 2 mM ADP, 2 mM KH_2PO_4, 50 mM tricine and 80–100 μg chlorophyll, at pH 8.5. Uncoupling activity was usually expressed as the concn required to reduce by 50% the ratio of electron flow (ferricyanide reduction) to ATP synthesis (depletion of Pi). Alternatively the percentage uncoupling at a known concn was measured.

Studies on pigment levels in pumpkin cotyledons. Cotyledons of pumpkin (*Cucurbita pepo* cv Mammoth) were germinated and prepared as described in ref. [27]. They were placed flatside down in pairs in 5 ml of an aq. soln of compound (10 μM) or H_2O in a Petri dish (dia 50 mm) for 4 days under continuous illumination from a 15 W fluorescent tube at 20°. They were weighed before and after treatment so that a growth index (increase in wt/initial wt) could be calculated. They were then extracted in 80% Me_2CO and the chlorophyll measured [28]. The relative chlorophyll accumulation (Table 5) was the total chlorophyll/growth index, normalized to 100% for the controls. Total carotenoids were separated from 80% Me_2CO extracts of cotyledons by known methods [29]. They were applied in a small vol. of petrol to small columns of freshly 'de-activated' Al_2O_3 (in Pasteur pipettes) and these were successively eluted with petrol, 0.5% Et_2O in petrol and 20% C_6H_6 in Et_2O. All samples were handled simultaneously so that the emphasis was on identical treatment of extracts rather than absolute recoveries. The first fraction had the absorption spectrum of β-carotene and the third fraction that of lycopene [30]. The second was γ- or δ-carotene, but since the fluctuations in its level were modest we did not extensively investigate variations in this peak. The tissue contents in Fig. 1 are expressed as a percentage of the amount recovered from untreated tissue in the same expt. Only one set of data is shown but the expt has been repeated with essentially the same result.

Growth of plants. Test plants used in these expts were grown at 20 ± 1° in John Innes No. 1 compost in a 14 hr light period of 8000 lx provided by white fluorescent tubes.

Estimation of concn of compound **15** *in treated peas.* Trays of peas were sprayed with **15** in 50% aq. Me_2CO at the rate of 0.7 kg/ha. The green parts of the plants were washed with C_6H_6 to remove any compound dissolved in surface waxes and homogenized in EtOAc. The solids were removed by centrifugation and the compound extracted with HCl (2 N). The aq. layer was neutralized and the compound re-extracted into Et_2O. The Et_2O layer was dried ($MgSO_4$), evapd to dryness under red. pres. and the residue dissolved in a known vol. of Me_2CO for assay by GLC (2.7 m 2% OV17 on Gas-Chrom-Q 80–100 mesh at 240° using a FID) relative to known quantities of standards.

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