SYNTHESIS AND PESTICIDAL EVALUATION OF PHENAZINES

I.—Halophenazines

By B. CROSS, C. L. DUNN, D. H. PAYNE and J. D. TIPTON

The synthesis and biological activity of twenty-six halophenazines is described. The Wohl-Aue reaction and the new method for cyclisation of 2-nitrodiphenylamines by oleum were found to be the most convenient methods of synthesis.

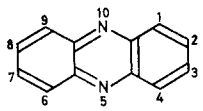
Phytotoxicity of a characteristic type was higher in foliar spray than in pre-emergence tests. Chlorine substitution appeared to confer higher activity than other halogen substituents, and in the chlorophenazines activity decreased with increasing substitution. Both 1- and 2-chlorophenazine were highly effective herbicides.

Acaricidal and fungicidal activity showed similar responses to structural changes, and optimum activity, together with a low level of phytotoxicity, was reached with 1,4-dichlorophenazine.

Introduction

Naturally occurring phenazine compounds have been isolated from bacteria and several of these affect the growth and viability of a wide range of micro-organisms.[1] Indications of potential pesticidal use have until recently been restricted to nematicidal activity[2] and, for phenazine itself, a low level of insecticidal activity[3] coupled with foliar scorch symptoms.[4] The closely related quinoxalines have been developed as pesticides; for example oxythioquinox, (1,3-dithiolo(2,3-b)5-methylquinoxaline-2-one),[5] as an acaricide and powdery mildew fungicide. It was of interest therefore to design and synthesise phenazines as potential pesticides.

The numbering of the phenazine nucleus used in this report is shown below.



Experimental

Chemical synthesis

All melting points are uncorrected (see Table I).

1,2,4-Trichlorophenazine (17) (Method A)

Aniline (20 g), 2,3,5-trichloronitrobenzene (52 g), powdered potassium hydroxide (120 g) and dry toluene (500 ml) were stirred vigorously and heated under reflux for 2 hours. The water eliminated during the reaction was removed by means of a Dean Stark apparatus. The hot mixture was filtered and the filtrate was evaporated to dryness to give a solid, m.p. 182°. Purification by chromatography on neutral alumina using benzene–chloroform as eluant gave, after crystallisation from ethyl acetate, 1,2,4-trichlorophenazine (17), 17% yield, m.p. 185–186° (Analysis, Table I).

2-Chlorophenazine-10-oxide (25) (Method B)

To a stirred concentrated sulphuric acid solution (20 ml) of 4′-chloro-2-nitrodiphenylamine (6·8 g, 0·027 mole) maintained at 20° by external cooling, was added 28% oleum (25 ml). After five minutes, the reaction mixture was slowly poured into a vigorously stirred solution of water (2·5 l) and ethanol (300 ml) containing sodium bicarbonate (150 g). A yellow precipitate separated out; the total mixture was extracted with methylene chloride (2·5 l). After the methylene chloride had been dried over anhydrous sodium sulphate, the solution was evaporated under reduced pressure to give a brown solid (8·4 g). Crystallisation from ethanol gave 2-chlorophenazine-10-oxide (25), 4·05 g (64%) m.p. 176–177°. (Analysis, Table I.)

In a similar manner 2,8-dichlorophenazine-10-oxide m.p. 228–229° was prepared from 4,4′-dichloro-2-nitrodiphenylamine in 77% yield, 2,7-dichlorophenazine-5-oxide m.p. 236–240° from 4′,5-dichloro-2-nitrodiphenylamine in 54% yield, 1,7-dichlorophenazine-5-oxide, m.p. 197–198° in 80% yield from 4′,6-dichloro-2-nitrodiphenylamine and 2,3-dichlorophenazine-5-oxide m.p. 222–223° from 4,5-dichloro-2-nitrodiphenylamine in 5% yield.

Reduction of phenazine-N-oxides

In most examples of Method A and all of Method B, phenazine-*N*-oxides result. These have been reduced to the phenazine by a variety of procedures.

An aniline solution (14 l) of 2-chlorophenazine-5-oxide (1,264 g) was heated under reflux for 8 hours. Excess aniline was distilled off *in vacuo* to a volume of 2·5 l. On cooling, the product crystallised out. This solid was dissolved in benzene and filtered through a short alumina column to remove dark impurities. The solvent was removed. After being washed with light petroleum the product (3), 910 g (76%), m.p. 138–140° was obtained.

2-Chlorophenazine-5-oxide (30·3 kg, 131 moles) was suspended in glacial acetic acid (120 l). The mixture was heated to 40° and iron powder (5·9 kg, 80% iron, 105 g atoms) was slowly added, the temperature not being allowed to exceed 65°. After being stirred for a further hour, the solution was run into water (1,200 l) to precipitate the product. The solid was filtered off, washed successively with water, methanol and light petroleum ether (40–60°) to give 26·3 kg, 122·7 moles (93·5%), identical with 2-chlorophenazine obtained by other procedures.

TABLE I

Preparation and biological activity of halophenazines

	Phenazine	Yield[a], % (Method)	m.p. °C	Analysis, %	Phytotoxicity ratings[k] Post-emergence kg/ha 10	Post-emergence kg/ha 1	Pre-emergence kg/ha 10	Pre-emergence kg/ha 1	Acaricidal[l] activity TI	Fungitoxicity[m] category
1	1-Cl	18 (A)	122[i]	Found C 67·3 H 3·2 N 13·0 Cl 16·7 Req. C 67·5 H 3·3 N 13·1 Cl 16·6	9	7	8	0	300	B
2	2-F	2 (A)	175–176	Found C 67·9 H 3·8 N 12·9 Req. C 67·3 H 3·3 N 13·1	6	1	5	0	40	D
3	2-Cl	62 (B) 30 (A)	139–140[h]	Found C 67·3 H 3·1 N 13·1 Cl 16·8 Req. C 67·5 H 3·3 N 13·1 Cl 16·6	8	6	6	2	300	C
4	2-Br	— (A)	149–150[b]	—	5	3	2	0	400	C
5	2-I	[c]	169–170[c]	—	2	1	0	0	50	—
6	1,2-di-Cl	21 (A)	172–173	Found C 57·7 H 2·4 N 11·0 Cl 28·4 Req. C 57·9 H 2·4 N 11·4 Cl 28·5	6	2	1	0	80	B
7	1,3-di-Cl	1·25 (A)	189–190	Found Cl 27·2 Req. Cl 28·5	2	0	1	0	50	C
8	1,4-di-Cl	40 (A)	191–192	Found C 58·0 H 2·6 Cl 28·2 Req. C 57·9 H 2·4 Cl 28·5	3	1	1	0	450	A
9	1,6-di-Cl	3 (A)	265–266	Found C 57·9 H 2·5 N 11·3 Cl 28·3 Req. C 57·9 H 2·4 N 11·3 Cl 28·5	0	0	0	0	B	D
10	1,7-di-Cl	17 (B)	200–201	Found Cl 27·8 Req. Cl 28·5	0	0	0	0	C	B
11	1,8-di-Cl	4 (A)	219–220	Found C 58·3 H 2·4 N 11·2 Cl 28·4 Req. C 57·9 H 2·4 N 11·3 Cl 28·5	1	0	0	0	C	D
12	1,9-di-Cl	20 (A)	206·5–207·5	Found C 57·9 H 2·6 N 11·2 Cl 28·4 Req. C 57·9 H 2·4 N 11·3 Cl 28·5	2	0	1	0	C	B
13	2,3-di-Cl	0·25 (A) 5 (B)	246–247[j]	—	0	0	0	0	B	C
14	2,7-di-Cl	12 (B)	265·5–268[g]	—	1	0	0	0	B	C
15	2,8-di-Cl	62 (B)	230–231	Found C 57·6 H 2·4 N 11·1 Cl 28·4 Req. C 57·9 H 2·4 N 11·3 Cl 28·5	2	1	0	0	C	C
16	1,2,3-tri-Cl	1 (A)	202	Found C 50·8 H 1·9 Cl 37·7 Req. C 50·8 H 1·8 Cl 37·4	—	—	—	—	< 10	—
17	1,2,4-tri-Cl	17 (A)	185–186	Found C 50·9 H 1·9 N 9·9 Cl 37·8 Req. C 50·8 H 1·8 N 9·9 Cl 37·4	1	0	0	0	< 10	C
18	1,2,9-tri-Cl	1 (A)	204·5–205·5	Found C 50·7 H 1·9 Cl 37·3 Req. C 50·8 H 1·8 Cl 37·5	–	—	—	—	B	B
19	1,4,6-tri-Cl	0·5 (A)	215–216	Found C 50·5 H 1·8 Cl 37·2 Req. C 50·8 H 1·8 Cl 37·5	1	0	1	0	B	B
20	1,4,7-tri-Cl	17 (A)	220–221	Found C 51·1 H 2·2 N 9·6 Cl 37·4 Req. C 50·8 H 1·8 N 9·9 Cl 37·5	0	0	1	0	C	D
21	1,2,3,4-tetra-Cl	90[d]	235[d]	Found C 45·5 H 1·5 N 9·5 Cl 44·4 Req. C 45·4 H 1·3 N 8·8 Cl 44·5	0	0	0	0	B	D
22	1,4,6,8-tetra-Cl	— (A)	210[e]	—	0	0	0	0	C	—
23	1-Cl; 5-O	87	158–158·5	Found C 62·4 H 3·4 N 11·7 Cl 15·4 Req. C 62·5 H 3·0 N 12·1 Cl 15·5	6	4	3	1	100	—
24	2-Cl; 5-O	30 (A)	176–177	Found C 62·5 H 3·0 N 12·0 Cl 15·2 Req. C 62·5 H 3·0 N 12·1 Cl 15·4	6	6	4	0	20	—
25	2-Cl; 10-O	64 (B)	174–176	Found C 62·6 H 3·0 N 12·1 Cl 15·4 Req. C 62·5 H 3·0 N 12·1 Cl 15·5	6	5	5	0	C	—
26	2-Cl; 5,10-di-O	95[f]	182[f]	—	5	2	4	1	B	—

[a] If the synthesis involves an N-oxide intermediate, the yield refers to the overall yield of N-oxide preparation and its reduction to the phenazine.
[b] Ref. 8 m.p. 149–150° [c] Ref. 8 m.p. 169–170°, prepared by the diazotisation of 2-aminophenazine and its reaction with cuprous iodide.
[d] Ref. 9 m.p. 235°, prepared from condensation of *o*-phenylenediamine and tetrachloro-*o*-benzoquinone. [e] Ref 10 m.p. 210°.
[f] Prepared by oxidation of compound 3, 24 or 25 with 30% H_2O_2 in glacial acetic acid at 50°C, lit., m.p. 190–191°.
[g] Ref. 12 m.p. 265–266°. — indicates data not available. [h] Ref. 1 m.p. 140°. [i] Ref. 1 m.p. 122–123°. [j] Ref. 15 m.p. 250–251°
[k] Mean phytotoxicity scores for all seven test species, rated on a 0–9 scale (0 = no effect, 9 = killed).
[l] TI (toxicity index) = $\frac{LC_{50} \text{ of methyl parathion}}{LC_{50} \text{ of phenazine}} \times 100$
B, C = incomplete kill and no appreciable kill in the preliminary screen
[m] Inhibition of cucumber powdery mildew: A = 90–100% inhibition at 50 ppm or equal to Karathane in the same test
B = 90–100% inhibition at 100 ppm or half as active as Karathane in the same test
C = 90–100% inhibition at 300 ppm
D = 90–100% inhibition at 1,000 ppm, or > 50% inhibition at 300 ppm
E = Inactive at the highest concentration tested (300 or 1000 ppm)

Biological evaluation

Herbicide screen

The following species were used for measuring the phytotoxicity of each chemical: maize (*Zea mays*), oat (*Avena sativa*), ryegrass (*Lolium perenne*), pea (*Pisum sativum*), linseed (*Linum usitatissimum*), mustard (*Sinapis alba*), and sugar-beet (*Beta vulgaris*).

For post-emergence application, batches of each of the seven test species were grown to a seedling stage with one or two true leaves. In the pre-emergence test, seeds of the same seven species were sown in bands in John Innes potting medium with a flint grit covering in plastic dishes, and watered shortly before treatment.

Each chemical was dissolved or suspended in 50% acetone in water containing 1·25% Triton X-155 wetting agent. This was sprayed on to the seedling foliage or seed trays by a moving-belt spraying apparatus.

At the end of the test period (7 days for the foliar spray test and eleven days for the pre-emergence test) the results were recorded visually. Phytotoxicity was rated on a 0–9 scale (0 = no effect, 9 = killed). The mean scores for all seven species treated with 1 and 10 kg/ha are shown in Table I.

Acaricide screen

Primary screening of the phenazines was carried out against 7–10 day-old glasshouse red spider mites (*Tetranychus telarius* L.) reared on French-bean plants.

For a preliminary test 0·7% w/v of each chemical was formulated as a solution or fine suspension in 20% acetone in water containing 0·05% Triton X-100 wetting agent. Further dilutions were used to determine dosage-mortality curves. Methyl parathion, formulated as above and applied over the range 0·05–0·005%, was used as the standard.

Discs cut from French-bean leaves and supported on moist filter paper were sprayed by a logarithmic spraying apparatus at a rate equivalent to 40 gal/acre. After being sprayed the discs were left for a ½–1 hour drying period, and then infested with 10 mites. Twenty-four hours after infestation—during which time the discs were kept under normal glasshouse conditions—the mites were examined for mobility. The number of immobile, dead and moribund mites was recorded and, in the preliminary screen, the compounds were rated A for complete or nearly complete kill, B for incomplete kill, and C for no appreciable kill. Compounds rated A in the preliminary screen underwent LC_{50} determinations. The toxicity of each active phenazine was compared with that of the standard and expressed as the toxicity index (TI), (Table I) where:

$$TI = \frac{LC_{50} \text{ of methyl parathion}}{LC_{50} \text{ of phenazine}} \times 100$$

Fungicide screen

As preliminary fungicide screens had shown that the phenazines possess significant activity only against powdery mildew fungi, the disease selected for structure–activity studies was cucumber powdery mildew (*Erysiphe cichoracearum*). Cucumber seedlings (cv. Butcher's Disease Resister) were grown in 3½ in pots of John Innes potting medium until two true leaves were fully expanded.

Each chemical was dissolved or suspended in 5 or 10% acetone in water containing 0·005% Triton X-100 wetting agent. Preliminary tests were carried out with concentrations of 1000 ppm and 300 ppm; active compounds were tested further at 100 and 50 ppm. Karathane (25% dinocap WP) was included in each test as a commercial standard. These aqueous preparations were sprayed, by a DeVilbiss Aerograph hand sprayer, on to the upper surfaces of two leaves per plant, and three plants were sprayed per treatment. The leaves were allowed to dry and then inoculated by dusting-on conidiospores of *Erysiphe cichoracearum* from infected leaves. Readings were made after 7 to 10 days' incubation in normal glasshouse conditions. The powdery mildew infection on each leaf was rated on a visual scale based on percentage of leaf area infected, where 0 = no infection, 1 = 1–10% infected, 2 = 11–20% infected..... 10 = 91–100% infected.

The fungicidal (or fungistatic) activity (Table I) is expressed by the following categories:

A = 90–100% reduction in infected area at 50 ppm, or equal to Karathane in the same test;
B = 90–100% reduction in infected area at 100 ppm, or half as active as Karathane in the same test;
C = 90–100% reduction in infected area at 300 ppm;
D = 90–100% reduction in infected area at 1000 ppm (or > 50% at 300 ppm); and
E = Inactive at the highest concentration tested (300 or 1000 ppm).

Results

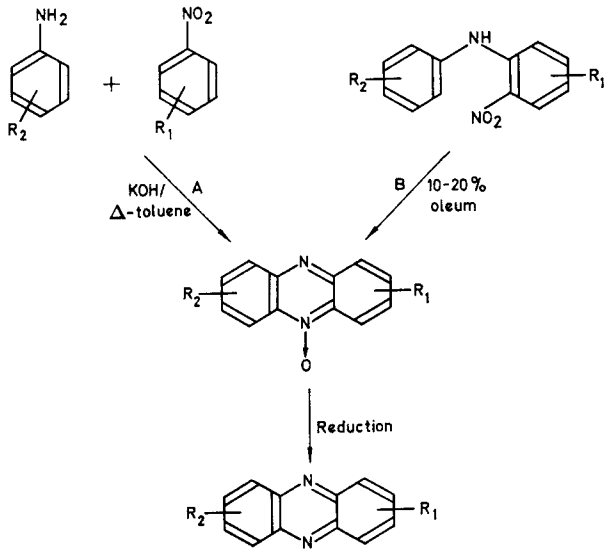
Synthesis of halophenazines

Method A

Halophenazines were most conveniently prepared by the Wohl-Aue synthesis,[6] which is the reaction of an aniline with nitrobenzene in the presence of powdered potassium hydroxide in a solvent such as dry toluene. Yields were generally low but were somewhat better if the halogen group was present in the nitrobenzene rather than the aniline ring. A phenazine-*N*-oxide was isolated except when the expected product had a substituent *ortho* to the *N*-oxide nitrogen, in which case the phenazine was obtained directly e.g. 1,4-dichlorophenazine (8). The halophenazine-*N*-oxides were readily reduced in 80–95% yields to the corresponding halophenazines by either heating under reflux with aniline for several hours or by iron–acetic acid reduction at 40°.

Method B

The new method for cyclisation of halogenated 2-nitrodiphenylamines in the presence of an excess of 10–20% oleum has been developed[7] as a procedure leading to halophenazine-*N*-oxides. It is particularly effective when halogen substitution is in the ring containing no nitro-group. For example 4′,6-dichloro-2-nitrodiphenylamine afforded 1,7-dichlorophenazine-5-oxide in 80% yield, which on reduction by iron–acetic acid gave 1,7-dichlorophenazine.



Phytotoxicity

The symptoms developed by plants treated with phenazines were distinct and characteristic. In particular broad-leaved species (e.g. linseed and mustard) were severely deformed, the leaf margins curling upwards. In the foliar spray test acute necrosis was also caused and, in general, activity was higher in this test than in the pre-emergence soil spray. Not all the phenazines were phytotoxic at the maximum dosage (10 kg/ha) and the level of phytotoxicity varied with the substituent and the substitution pattern. Chlorophenazines appeared to be the most phytotoxic as exemplified by the 2-halophenazine series in which 2-Cl > 2-F $\geqslant$ 2-Br > 2-I. The following observations regarding structure–activity relationships were therefore restricted to chloro substituents. Mono-substitution conferred the highest degree of phytotoxicity, and both 1-chloro and 2-chlorophenazine were highly active. Activity was reduced as the number of substituents was increased, giving the general order mono > di > tri > tetra, although there was considerable variation in activity between positional isomers, e.g. 1,2-, 1,3- and 1,4-dichlorophenazines were all moderately active but 2,3-dichlorophenazine was inactive.

Substitution into both rings conferred less activity than substitution in one ring so that the six examples of dichloro substitution in both rings (9–12, 14, 15) were generally less active than the four examples of dichloro substitution in one ring (6–8, 13). Trichloro substitution, whether in one or both rings, resulted in such low levels of phytotoxicity that no obvious patterns emerged, while tetrachloro substitution rendered the compound inactive (21 and 22).

Chloro-*N*-oxides (23–26) exhibited lower levels of phytotoxicity than the parent phenazines. Activity appeared to be about the same whether substitution was in the 5 or 10 position and was not markedly decreased by di-substitution in both positions.

Acaricidal activity

In the 2-halosubstituted series, the chloro-(3) and bromophenazines (4) showed a high level of acaricidal activity, whereas the fluoro-(2) and iodophenazines (5) were significantly less active. The monochlorophenazines (1 and 3) appeared equally active but the activity of the dichlorophenazines (6–8, 13) with halogen in only one ring varied considerably with positional isomers, in the order 1,4 > 1,2 > 1,3 > 2,3. Trichlorophenazines (16 and 17) were considerably less active, whilst the tetrachloro compound (21) was virtually inactive.

Chlorine substitution in both rings (eg. 9–12) caused almost complete loss of acaricidal activity. Mono-*N*-oxides (23–25) were acaricidal, but at a lower level than their parent phenazines (1,3), the di-*N*-oxide (26) was almost inactive.

Fungitoxicity

In the control of cucumber powdery mildew both 2-chloro- and 2-bromophenazine (3, 4) were more active than 2-fluorophenazine (2), but 1-chlorophenazine (1) was significantly more effective. Dichloro substitution in one ring (6–8, 13) conferred activity which closely paralleled the acaricidal activity. Dichlorophenazines having one substituent in the 1-position were at least as active as 1-chloro-phenazine (1) when the second substituent was in the 2-,4-,7- or 9-position, but were less active than 1-chlorophenazine when the second substituent was in the 3-,6- or 8- position. 1,4-Dichlorophenazine was distinctly more effective than 1-chlorophenazine, with much less phytotoxicity. Dichlorophenazines with both chlorines β to the nitrogens (13–15) were equal in effect to 2-chlorophenazine (3). Further substitution in one or both benzo-rings (17–21) produced no general improvement on the activity of the corresponding dichlorophenazines.

Discussion

The monohalophenazines possess a high degree of general biological activity and could have the same metabolic site of action in the three types of organism. 1-Chlorophenazine is very toxic to plants, mites and fungi, but with other monohalophenazines there appears to be no general pattern relating phytotoxicity, acaricidal activity and fungitoxicity. Possibly the electronic and steric properties of each halogen influence the penetration of the three different biological membranes differently, resulting in some specificity. However, the phytotoxicity is always high enough to make the fungicidal and acaricidal members worth little further consideration for use as plant protectants.

The dichlorophenazines, which are generally less phytotoxic, show some relationship between toxicity to the three organisms and the positions of substitution. This is best shown when one chlorine is in the 1-position, and when the position of the second chlorine substituent is related to arbitrary measures of each of the biological activities.

The fungicidal activity varies with the consecutive substitution positions. This is paralleled up to 6-chloro by the acaricidal activity which then decreases to zero. The variation is also detectable with the phytotoxicity but as part of a trend decreasing to a minimum at 6-chloro and increasing again at 8-chloro and 9-chloro. This trend is shown by the phytotoxicity parallels and possibly reflects the trend in the polarity in the dichlorophenazines reported by Morita.[13]

A similar variation is found in the phytotoxicity and acaricidal activity when one chlorine is in the 2-position, and the position of the second chlorine is again plotted against biological activity. Excluding cases where penetration is presumed to be the limiting factor the response shown by the three types of organism to the positions of substitution indicates a common metabolic mode of action.

Tri- and tetra- substitution reduces the phytotoxicity and

acaricidal activity to a low level. The fungicidal activity of the tri- and tetra-chlorophenazines was generally less than that of related dichlorophenazines. These observations suggest that substitution in excess of two chlorines leads to hindrance of the chemical either at external biological membranes or at the site of action. Oxidation of one or both nitrogens successively reduces the biological activity. It seems probable that the biological action of the phenazines involves single-electron oxidation–reduction reactions at the nitrogen atoms,[14] so that N-oxidation can be considered to block the site of biological activity.

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Shell Research Ltd.,
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