Synthesis and Fungicidal Activity of Alicyclic Diamines

Neil D. Havis and Dale R. Walters*

Department of Plant Science, SAC, Auchincruive, Nr Ayr KA6 5HW, United Kingdom

William P. Martin, Fiona M. Cook, and David J. Robins

Department of Chemistry, The University, Glasgow G12 8QQ, United Kingdom

As part of an ongoing program of work on polyamine analogues, a number of alicyclic diamines were synthesized and examined for fungicidal activity. The alicyclic diamine 1,2-bis(aminomethyl)-4,5-dimethylcyclohexa-1,4-diene (BAD), synthesized as the dihydrochloride salt, controlled the important crop pathogen *Erysiphe graminis* f.sp *hordei*. Greatest control of *E. graminis* was achieved when BAD was applied 2 days postinoculation. The alicyclic diamines *trans*-4,5-bis(aminomethyl)-1,2-dimethylcyclohex-1-ene (*trans*-BAD) and the *cis*-isomer (*cis*-BAD), as well as 1,2-bis(aminomethyl)cyclopentene (BACP) and *trans*-1,2-bis(dimethylaminomethyl)cyclobutane (TCCBM), were also synthesized as their dihydrochloride salts. *trans*-BAD was found to possess greater fungicidal activity than the *cis*-isomer against *E. graminis*, while BACP and TCCBM both gave >80% control of powdery mildew infection. Since the powdery mildew fungus cannot be grown axenically, the effects of the alicyclic diamines on polyamine metabolism were examined using the oat leaf stripe pathogen, *Pyrenophora avenae*. BAD and derivatives had little effect on polyamine metabolism in the fungal pathogen *P. avenae*. It seems clear, therefore, that the antifungal activity of these derivatives may not be associated with altered polyamine metabolism.

Keywords: *Polyamines; alicyclic diamines; fungicide; powdery mildew*

INTRODUCTION

Inhibitors of polyamine biosynthesis have been shown previously to give effective control of biotrophic fungal pathogens, such as rusts and powdery mildews (Rajam et al., 1985; Walters, 1986; West et al., 1988). This early work centered mostly on the use of enzyme-activated irreversible inhibitors, e.g. α-difluoromethylornithine (DFMO), an inhibitor of the polyamine biosynthetic enzyme ornithine decarboxylase (ODC) (Metcalf et al., 1978). In plant pathogenic fungi, DFMO produced a depletion in the intracellular concentrations of putrescine and spermidine (Foster and Walters, 1990). However, an alternative method of polyamine perturbation has been demonstrated using polyamine analogues (Porter and Sufrin, 1986). As a result, a variety of polyamine analogues have been shown to alter polyamine metabolism in tumor cells leading to pronounced antiproliferative effects. More recently, ketoputrescine, a commercially available putrescine analogue, was shown to possess fungicidal properties (Foster and Walters, 1993). In previous papers, we have described the fungicidal activity of a number of aliphatic putrescine analogues (Havis et al., 1994a,b). This paper describes the synthesis, as their salts, and fungicidal evaluation of a number of alicyclic diamines.

EXPERIMENTAL PROCEDURES

Synthesis of 1,2-Bis(aminomethyl)-4,5-dimethylcyclohexa-1,4-diene Dihydrochloride (BAD) (1). To a solution of diisobutyl aluminum hydride (DIBAL) in dichloromethane (1.0 M, 45 mL, 0.045 mol) cooled to ice bath temperature under nitrogen was added dimethyl 4,5-dimethylcyclohexa-1,4-diene-1,2-dicarboxylate (2.24 g, 0.01 mol) (Kucherov and Grigoreva, 1961) in dry dichloromethane (30 mL) over 30 min. The resultant solution was stirred for a further 60 min at this temperature, after which time methanol (10 mL) was added. The mixture was allowed to warm to room temperature and filtered through a Celite pad, and the filtrate was concentrated *in vacuo* to give an oil. Crystallization from ethyl acetate/petroleum ether (40−60 °C) gave 1,2-bis(hydroxymethyl)-4,5-dimethylcyclohexa-1,4-diene in 52% yield: mp 146−148 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 4.16 (s, 4H), 2.76 (s, 4H), 1.65 (s, 6H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 132.4 (C), 122.8 (CH_3), 62.0 (CH_2), 36.7 (CH_2), 18.0 (CH_3); IR (KBr disk) 3280, 2900, 2870, 2700, 1050, 1010, 990 cm^{-1}; MS (*m*/*z*) 168 (M^+). Anal. Calcd for $C_{10}H_{16}O_2$: C, 71.43; H, 9.52%. Found: C, 70.67; H, 9.43%.

To a solution of hydrazoic acid (1.0 M) in benzene (24 mL) were added 1,2-bis(hydroxymethyl)-4,5-dimethylcyclohexa-1,4-diene (1.68 g, 0.01 mol) in dry tetrahydrofuran (THF; 20 mL) and diisopropyl azodicarboxylate (2.52 g, 0.025 mol) in dry THF (10 mL). To this stirred solution was added triphenylphosphine (10.48 g, 0.05 mol) in dry THF (35 mL) over 1 h. The mixture was then stirred at 50 °C for a further 7 h, and then water (5 mL) was added. The mixture was allowed to cool and then partitioned between hydrochloric acid (1 M, 100 mL) and CH_2Cl_2 (100 mL). The aqueous layer was further washed with CH_2Cl_2 (2 × 80 mL), and the water was removed *in vacuo* to give a brown solid, which was dissolved in ethanol and precipitated with ether to give 1,2-bis(aminomethyl)-4,5-dimethylcyclohexa-1,4-diene dihydrochloride in 32% yield: ^{1}H NMR (200 MHz, D_2O) δ 3.76 (s, 4H), 2.76 (s, 4H), 1.64 (s, 6H); ^{13}C NMR (50 MHz, D_2O) δ 140.2 (C), 123.2 (C), 40.2 (CH_2), 36.0 (CH_2), 19.6 (CH_3); IR (KBr disk) 3420, 3010, 2920, 1660, 1640, 1507, 1480, 1450, 1380, 1110 cm^{-1}; MS (*m*/*z*) 166 (M^+ − 2HCl). Anal. Calcd for $C_{10}H_{20}N_2Cl_2$: C, 50.21; H, 8.37; N, 11.72%. Found: C, 50.46; H, 8.17; N, 11.56%.

Synthesis of *trans*-4,5-Bis(aminomethyl)-1,2-dimethylcyclohexene Dihydrochloride (*trans*-BAD) (2). To a suspension of lithium aluminum hydride (1.52 g, 0.04 mol) in dry THF (60 mL) at 0 °C was added dimethyl *trans*-1,2-dimethylcyclohexene-4,5-dicarboxylate (Le Coq and Levas, 1966) (2.26 g, 0.01 mol) in dry THF (40 mL) over 30 min. The resultant suspension was stirred for another hour at 0 °C, and then saturated sodium sulfate (ca. 5 mL) was added dropwise.

* Author to whom correspondence should be addressed.

S0021-8561(95)00772-2 CCC: $12.00

The solution was filtered, and the filtrate was concentrated to give an oil, which on purification gave *trans*-4,5-bis-(hydroxymethyl)-1,2-dimethylcyclohexene in 36% yield: mp 102−104 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 3.61 (m, 4H), 3.48 (m, 2H), 1.76 (m, 4H), 1.53 (s, 6H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 124.6 (C), 66.2 (CH_2), 40.7 (CH), 35.2 (CH_2), 18.6 (CH_3); IR (thin film) 3300, 2890, 2880, 1410, 1380, 1080, 1030 cm^{-1}; MS (m/z) 170 (M^+). Anal. Calcd for $C_{10}H_{18}O_2$: C, 70.59; H, 10.59%. Found: C, 70.65; H, 10.68%.

trans-4,5-Bis(Hydroxymethyl)-1,2-dimethylcyclohexene was treated with hydrazoic acid as described above to give a brown solid, which was recrystallized twice (ethanol/acetone) to give *trans*-4,5-bis(aminomethyl)-1,2-dimethylcyclohexene dihydrochloride is 22% yield: ^{1}H NMR (200 MHz, D_2O) δ 2.85 (m, 4H), 1.96 (m, 4H), 1.60 (m, 2H), 1.47 (s, 6H); ^{13}C NMR (50 MHz, D_2O) δ 123.3 (C), 42.8 (CH_2), 33.7 (CH), 30.8 (CH_2), 19.0 (CH_3); IR (KBr disk) 3450, 3030, 2900, 2850, 1610, 1500 cm^{-1}; MS (m/z) 168 ($M^+ - 2HCl$). Anal. Calcd for $C_{10}H_{20}N_2Cl_2$: C, 49.79; H, 9.13; N, 11.62%. Found: C, 49.58; H, 9.15; N, 11.52.

Synthesis of *cis*-4,5-Bis(aminomethyl)-1,2-dimethylcyclohexene Dihydrochloride (*cis*-BAD) (3). *cis*-1,2-Dimethylcyclohexene-4,5-dicarboxylic anhydride (Grummitt and Eudrey, 1960) was reduced with lithium aluminum hydride as described above to give a yellow oil of *cis*-4,5-bis(hydroxymethyl)-1,2-dimethylcyclohexene in 36% yield: mp 72−74 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 4.71 (s, 2H), 3.57 (m, 4H), 1.97 (m, 6H), 1.59 (s, 6H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 123.9 (C), 63.5 (CH_2), 38.4 (CH), 33.3 (CH_2), 19.0 (CH_3); IR (thin film) 3310, 2900, 1440, 1080, 1010, 690 cm^{-1}; MS (m/z) 152 ($M^+ - H_2O$). Anal. Calcd for $C_{10}H_{18}O_2$: C, 70.59; H, 10.59%. Found: C, 70.76; H, 10.76%.

Treatment of this product with hydrazoic acid as described above gave a brown solid, which was recrystallized (ethanol/acetone) to give *cis*-4,5-bis(aminomethyl)-1,2-dimethylcyclohexene dihydrochloride in 22% yield: ^{1}H NMR (200 MHz, D_2O) δ 3.95 (m, 4H), 1.99 (m, 2H), 1.93 (m, 4H), 1.52 (s, 6H); ^{13}C NMR (50 MHz, D_2O) δ 122.6 (C), 40.1 (CH_2), 31.4 (CH), 28.9 (CH_2), 19.4 (CH_3); IR (KBr disk) 3450, 3030, 2900, 2850, 1610, 1500 cm^{-1}; MS (m/z) 168 ($M^+ - 2HCl$). Anal. Calcd for $C_{10}H_{20}N_2$: C, 49.79; H, 9.13; N, 11.62%. Found: C, 49.85; H, 9.05; N, 11.25%.

Synthesis of 1,2-Bis(aminomethyl)cyclopentene Dihydrochloride (BACP) (4). Dimethylcyclopentene-1,2-dicarboxylate (McDonald and Reitz, 1972) was reduced with lithium aluminum hydride as described above to give an oil, which on vacuum distillation (120−130 °C, 0.05 mmHg) gave 1,2-bis-(hydroxymethyl)cyclopentene in 31% yield: mp 41−42 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 4.20 (s, H), 3.64 (bs, 2H), 2.47 (t, 4H), 1.85 (q, 2H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 129.6 (C), 62.6 (CH_2), 36.7 (CH_2), 23.0 (CH_2); IR (thin film) 3300, 2885, 2875, 1645, 1445, 1190 cm^{-1}; MS (m/z) 110 ($M^+ - H_2O$). Anal. Calcd for $C_7H_{12}O_2$: C, 65.63; H, 9.38%. Found: C, 65.60; H, 9.31%.

This diol was treated with hydrazoic acid (1.25 M) as described above to give a brown solid, which was recrystallized twice (ethanol/acetone) to give 1,2-bis(aminomethyl)cyclopentene dihydrochloride in 21% yield: ^{1}H NMR (200 MHz, D_2O) δ 3.63 (s, 4H), 2.35 (t, 4H), 1.79 (m, 2H); ^{13}C NMR (50 MHz, D_2O) δ 136.0 (C), 37.3 (CH_2), 34.8 (CH_2), 22.3 (CH_2); IR (KBr disk) 3434, 2951, 2264, 2212, 1601, 1473, 1406, 1385, 1120, 937 cm^{-1}; MS (m/z) 109 ($M^+ - 2HCl - NH_3$). Anal. Calcd for $C_7H_{16}N_2Cl_2$: C, 42.21; H, 8.04; N, 14.07%. Found: C, 42.13; H, 8.17; N, 13.91%.

Synthesis of *trans*-1,2-Bis(dimethylaminomethyl)cyclobutane Dihydrochloride (TCCBM) (5). The synthesis of the free base was carried out by modifying the general procedure of Quin et al. (1979). To a suspension of *trans*-1,2-cyclobutanedicarboxylic acid (0.5 g, 3.47 mmol) in benzene (5 mL) was added hexamethylphosphorus triamide (0.63 mL, 3.47 mmol) dropwise. The mixture was heated to ca. 50 °C, stirred for 30 min, and then cooled to room temperature; saturated sodium bicarbonate solution (5 mL) was then added. The layers were separated, and the aqueous layer was extracted with dichloromethane (3 × 20 mL). The organic extracts were combined, dried ($MgSO_4$), concentrated, and washed with hexane to yield *trans*-*N*,*N*,*N*′,*N*′-tetramethylcyclobutane-1,2-dicarboxamide (0.61 g, 89%): ^{1}H NMR (90 MHz, $CDCl_3$) δ 2.1 (4H, m), 2.85 (6H, s), 2.95 (6H, s), 3.7 (2H, m); IR (KBr disk) 3432, 2970, 2938, 1631 cm^{-1}; MS (m/z) 198 (M^+), 154, 126, 100, 72 (100%). HRMS calcd for $C_{10}H_{18}N_2O_2$: M^+ 198.1364 (23%). Found: 198.1369.

To a suspension of lithium aluminum hydride (0.25 g, 6.58 mmol) in anhydrous ether (10 mL) was added a solution of *trans*-*N*,*N*,*N*′,*N*′-tetramethylcyclobutane-1,2 dicarboxamide (0.6 g, 3.03 mmol) in dry THF (5 mL) at a rate which maintained gentle reflux. The mixture was then heated at reflux for 1 h and hydrolyzed by the cautious addition of water (1 mL), 15% sodium hydroxide solution (1 mL), and finally water (2 mL). The fine white precipitate was filtered off, washed with ether (3 × 10 mL), and discarded. The filtrate was concentrated to give a colorless oil (0.4 g, 77%): ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.57−2.46 (10H, m), 2.18 (12H, s); ^{13}C NMR (50 MHz, $CDCl_3$) δ 25.2 (CH_2), 38.5 (CH), 45.6 (CH_3), 65.4 (CH_2); IR (KBr disk) 3404, 2966, 2939, 2814, 2764, 1643, 1456 cm^{-1}; MS (m/z) 170 (M^+), 125, 84, 58 (100%). HRMS calcd for $C_{10}H_{22}N_2$: M^+ 170.1783. Found: 170.1784. The dihydrochloride was prepared by precipitation from a solution of the base in dry ether saturated with HCl gas.

Determination of Protectant and Curative Action of BAD against Powdery Mildew (*Erysiphe graminis* DC f.sp *hordei* Marchal) on Barley. Barley seedlings (*Hordeum vulgare* L. cv. Golden Promise) were grown in Fison's Levington compost in 36 cm seed trays. Plants were grown in a glasshouse under natural daylight supplemented for 16 h daily by 400 W mercury vapor lamps. The maximum temperature was 24 °C during the day and 9 °C at night. Plants at Zadoks growth stage 12 (second leaf unfolded) were sprayed before and after inoculation with the powdery mildew fungus. BAD and analogues were applied to plants in aqueous solution containing 0.1 mL L^{-1} Tween 20. Barley seedlings were sprayed to runoff with these solutions (usually 1 mM, unless stated otherwise) using a Shandon spray unit, 3 h before or 3 days after inoculation with pathogen. To study the effects of the time of application on infection, BAD was applied to barley seedlings 1, 2, and 5 days pre- or postinoculation with mildew. Plants were inoculated simply by dusting them with conidia of *E. graminis* f.sp *hordei*. Infection intensity was assessed 6−10 days after inoculation by estimating the percentage leaf area with pustules using a standard area diagram. Sporulation usually occurred about 6−7 days after inoculation.

Effects of *trans*-BAD and *cis*-BAD on Growth, Enzyme Activities, and Polyamine Concentrations in *Pyrenophora avenae*. Because of limited supplies of experimental test compounds, the effects of *trans*-BAD and *cis*-BAD on mycelial growth, polyamine concentrations, and the activities of ornithine decarboxylase (ODC; EC 4.1.1.17), *S*-adenosylmethionine decarboxylase (AdoMetDC; EC 4.1.1.50), and diamine oxidase (DAO; EC 1.4.3.6) were determined as described previously (Foster and Walters, 1990; Havis et al., 1994b).

RESULTS AND DISCUSSION

Synthesis of Alicyclic Diamines. The synthesis of compounds **1**−**3** was carried out by Diels−Alder cycloaddition reactions of 2,3-dimethylbutadiene with different alkenes to generate the six-membered rings, followed by reduction to the diols and conversion into the diamines (Figure 1). The five-membered diamine was made by Dieckmann cyclization of α,α′-dibromopimelate, followed by reduction and conversion into the diamine (**4**). The four-membered diamine (**5**) was prepared from *trans*-cyclobutane-1,1-dicarboxylic acid via the bis(diethylamide).

Fungicidal Activity of Alicyclic Compounds. BAD applied as a postinoculation spray provided very substantial control of powdery mildew (*E. graminis* f.sp *hordei*) on barley (93%; Table 1). Most effective control of *E. graminis* was obtained when BAD was applied 2 days postinoculation, indicating curative properties for this compound (Table 2). This effect was also observed

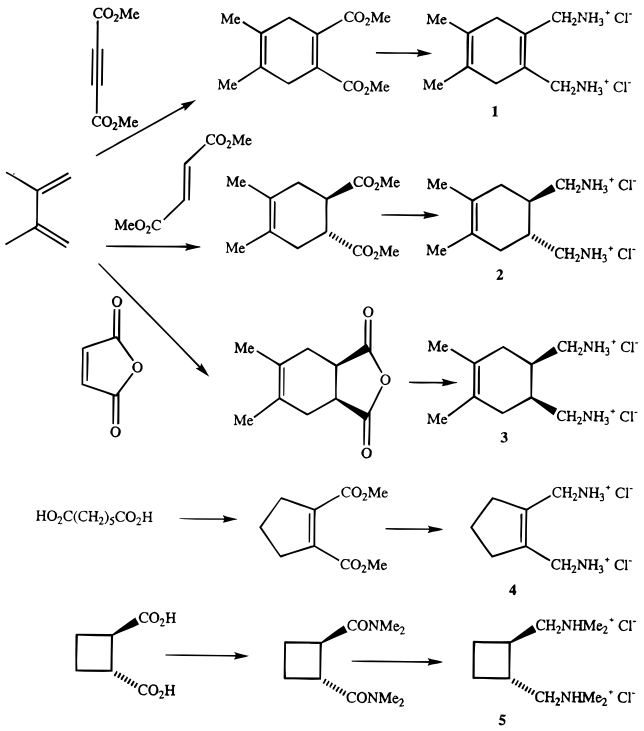

Figure 1. Chemical synthesis of alicyclic diamines.

Table 1. Effects of BAD and Derivatives on Powdery Mildew of Barley

treatment[a]	% leaf area infected	% disease control	lsd[c] ($P = 0.05$)
control	55.0		
BAD[b]	4.0	93	6.40
control	37.33		
trans-BAD	8.70	77	5.53
cis-BAD	14.20	62	6.01
control	51.08		
BACP	8.67	83	6.87
TCCBM	5.78	89	6.95

[a] Treatments were applied at 1 mM, i.e. 211, 211, 211, 199, and 243 mg L^{-1}, respectively, and were applied as postinoculation treatments. All compounds were used as salts (see Figure 2). [b] BAD, 1,2-bis(aminomethyl)-4,5-dimethylcyclohexa-1,4-diene dihydrochloride; *trans*-BAD, *trans*-4,5-bis(aminomethyl)-1,2-dimethylcyclohexene dihydrochloride; *cis*-BAD, *cis*-4,5-bis(aminomethyl)-1,2-dimethylcyclohexene dihydrochloride; BACP, 1,2-bis(aminomethyl)cyclopentene dihydrochloride; TCCBM, *trans*-1,2-bis(dimethylaminomethyl)cyclobutane dihydrochloride. [c] lsd, least significant difference.

Table 2. Effects of Timing of BAD Application on Powdery Mildew of Barley

treatment[a]	% leaf area infected	% disease control
control	28.9 ± 2.5	
5 days preinoculation	24.0 ± 0.9	17
2 days preinoculation	26.3 ± 4.3	9
1 day preinoculation	22.8 ± 2.5	21
1 day postinoculation	16.8 ± 2.0[b]	42
2 days postinoculation	8.9 ± 1.2[c]	70
5 days postinoculation	17.2 ± 1.9[b]	41

[a] Treatments were applied at 1 mM, i.e. 211 mg L^{-1}. [b,c] Significantly different from the control at $P \leq 0.005$ and ≤ 0.001, respectively.

with the synthetic putrescine analogues E-BED and E-TED (Havis et al., 1994a,b) and could be related to perturbation of polyamine biosynthesis in the germinating conidia on the leaf surface. The ODC inhibitor, DFMO, has already been shown to inhibit the germination of rust uredospores on the leaf surface (Rajam et al., 1989), and two putrescine analogues have been shown to inhibit appressorium formation by uredospores or *Uromyces viciae-fabae* (Reitz et al., 1995). Other alicyclic compounds also controlled infection by *E. graminis*. The most effective control was obtained with postinoculation treatments of TCCBM (89%; Table 1).

Table 3. Effect of *trans*-BAD[a] and *cis*-BAD[b] on the Growth of *P. avenae* in Liquid Culture

treatment	mean wt (g)	lsd ($P = 0.05$)
control	1.99	
trans-BAD	1.81	0.89
cis-BAD	2.09	0.89

[a] *trans*-BAD was used as the dihydrochloride at 211 mg L^{-1} (1 mM). [b] *cis*-BAD was used as the dihydrochloride at 211 mg L^{-1} (1 mM).

Table 4. Effect of *trans*-BAD and *cis*-BAD on Polyamine Concentrations in *P. avenae*

	polyamine concn (μmol g^{-1} fresh wt)			
treatment[a]	putrescine	cadaverine	spermidine	spermine
control	147.25 ± 20.99	54.02 ± 19.64	81.53 ± 8.12	86.87 ± 4.75
trans-BAD	126.01 ± 12.10	37.99 ± 7.34	72.79 ± 9.10	98.35 ± 5.69
cis-BAD	176.74 ± 46.30	34.03 ± 4.09	49.04 ± 6.15	118.48 ± 5.62
lsd ($P = 0.05$)	97.80	41.91	33.93	17.77

[a] *trans*-BAD and *cis*-BAD were both used as their dihydrochlorides at 211 mg L^{-1} (1 mM).

Table 5. Effect of *trans*-BAD and *cis*-BAD on Enzyme Activities in *P. avenae*

A. Enzyme Activity [pmol of CO_2 (mg of protein)$^{-1}$ h^{-1}]

treatment[a]	ODC	lsd ($P = 0.05$)	AdoMetDC	lsd ($P = 0.05$)
control	1.26 ± 0.38		62.53 ± 23.90	
trans-BAD	2.18 ± 0.55	1.66	22.12 ± 12.47	67.54
cis-BAD	1.63 ± 0.40	1.54	15.77 ± 2.76	72.95

B. Enzyme Activity [pmol of product (mg of protein^{-1}) h^{-1}]

treatment[a]	DAO	lsd ($P = 0.05$)
control	66.48 ± 0.95	
trans-BAD	50.50 ± 5.20	20.11
cis-BAD	44.61 ± 3.15	21.73

[a] *trans*-BAD and *cis*-BAD were both used as their dihydrochlorides at 211 mg L^{-1}.

Two isomers, *trans*-BAD and *cis*-BAD, exhibited differing fungicidal activities against *E. graminis* infection. Thus, *trans*-BAD reduced mildew infection by 77% (Table 1), while *cis*-BAD gave only 62% control of mildew infection (Table 1). These differences, however, were not significant. Similar effects were observed in previous work in which the synthetic putrescine analogue *trans*-1,4-diaminobut-2-ene (E-BED) was found to possess greater fungicidal activity than the *cis*-isomer, Z-BED (Havis et al., 1994a). The mechanism underlying this difference in fungicidal activity is not known.

Since powdery mildew cannot be grown axenically, the effects of these two isomers on polyamine biosynthetic and catabolic enzymes and polyamine levels were examined in the oat stripe pathogen, *P. avenae*. The alicyclic compound produced little effect on growth or polyamine metabolism in this fungus (Tables 3−5). This contrasts with the effects of aliphatic diamines, which have been shown to perturb polyamine biosynthesis (Havis et al., 1994a,b). Nevertheless, these authors suggested that the aliphatic diamines did not deplete intracellular polyamine concentrations suf-

ficiently to account for the observed antifungal activity. A similar situation exists in the present work. It is important to remember, however, that effects of BAD and derivatives on polyamine metabolism in developing germlings of *E. graminis* cannot be ruled out. Since the pathogen cannot be cultured axenically, the mode of action of BAD against this fungus is not going to be resolved easily.

It is interesting to note that a number of bis(benzyl)-polyamine analogues have been shown to be potent inhibitors of two strains of the human malaria parasite *Plasmodium falciparum in vitro* (Bitonti et al., 1989). These analogues were found to be 1000 times more potent than the free amine analogues. It was suggested that these compounds may not only repress polyamine biosynthesis but exert cytotoxic effects by binding directly to DNA and subsequently disrupt macromolecular synthesis. It would be useful to determine whether BAD and its derivatives disrupt macromolecular synthesis in *P. avenae.*

ACKNOWLEDGMENT

We are grateful to Dr. R. A'Court of BTG for advice and guidance.

LITERATURE CITED

Bitonti, A. J.; Bush, T. L.; McCann, P. P. Regulation of polyamine biosynthesis in rat hepatoma (HTC) cells by a bisbenzyl polyamine analogue. *Biochem. J.* **1989**, *257*, 769−774.

Foster, S. A.; Walters, D. R. The effects of polyamine biosynthesis inhibitors on mycelial growth, enzyme activity and polyamine levels in the oat infecting fungus *Pyrenophora avenae. J. Gen. Microbiol.* **1990**, *136*, 233−239.

Foster, S. A.; Walters, D. R. Fungicidal activity of the polyamine analogue, keto-putrescine. *Pestic. Sci.* **1993**, *37*, 267−272.

Grummitt, O.; Eudrey, A. L. Relative reactivity of maleic anhydride and sulfur dioxide in 1,4-addition reactions to 2,3-dimethyl-1,3-butadiene. *J. Am. Chem. Soc.* **1960**, *82*, 3614.

Havis, N. D.; Walters, D. R.; Foster, S. A.; Martin, W. P.; Cook, F. M.; Robins, D. J. Fungicidal activity of the synthetic putrescine anaolgue (*E*)-1,4-diaminobut-2-ene. *Pestic. Sci.* **1994a**, *41*, 61−69.

Havis, N. D.; Walters, D. R.; Martin, W. P.; Cook, F. M.; Robins, D. J. Fungicidal activity of three putrescine analogues. *Pestic. Sci.* **1994b**, *41*, 71−76.

Kucherov, V. F.; Grigoreva, N. Y. Conjugation factors in cyclic systems. *Zh. Obshch. Khim.* **1961**, *31*, 447.

Le Coq, A.; Levas, E. Aniotropic rearrangements of 4,4,4-trichlorocrotonaldehyde. *C. R. C* **1966**, *262*, 574.

McDonald, R. N.; Reitz, R. R. Strained ring systems. XII. *J. Org. Chem.* **1972**, *37*, 2418.

Metcalf, B. W.; Bey, P.; Danzin, C.; Jung, M. J.; Casara, P.; Vevert, J. P. Catalytic irreversible inhibition of mammalian ornithine decarboxylase (EC 4.1.1.17) by substrate and product analogues. *J. Am. Chem. Soc.* **1978**, *100*, 2551−2553.

Porter, C. W.; Sufrin, J. R. Interference with polyamine biosynthesis and/or function by analogs of polyamines or methionine as a potential anticancer chemotherapeutic strategy. *Anticancer Res.* **1986**, *6*, 525−542.

Quin, L. D.; Leimert, J.; Middlemas, F. D.; Miller, R. W.; McPhail, A. T. 3,8-Thionanedione 1,1-dioxide, synthesis and solid-state conformation. *J. Org. Chem.* **1979**, *44*, 3496−3500.

Rajam, M. V.; Weinstein, L. H.; Galston, A. W. Prevention of a plant disease by specific inhibition of fungal polyamine biosynthesis. *Proc. Natl. Acad. Sci. U.S.A.* **1985**, *82*, 6874−6878.

Rajam, M. V.; Weinstein, L. H.; Galston, A. W. Inhibition of uredospore germination and germ tube growth by inhibitors of polyamine metabolism in *Uromyces phaseoli* L. *Plant Cell Physiol.* **1989**, *30*, 37−41.

Reitz, M.; Walters, D. R.; Moerschbacher, B.; Robins, D. J. The effects of two synthetic putrescine analogues on germination and appressorium formation by uredospores on *Uromyces viciae-fabae* on artificial membranes. *Lett. Appl. Microbiol.* **1996**, *21*, 285−287.

Walters, D. R. The effects of a polyamine biosynthesis inhibitor on infection of *Vicia faba* L. by the rust fungus, *Uromyces viciae-fabae* (Pers.) Schroet. *New Phytol.* **1986**, *104*, 613−19.

West, H. M.; Walters, D. R. The effects of polyamine biosynthesis on infection of *Hordeum vulgare* L. by *Erysiphe graminis* f.sp. *hordei* Marchal. *New Phytol.* **1988**, *110*, 193−200.

Received for review November 22, 1995. Revised manuscript received July 9, 1996. Accepted July 16, 1996.[⊗] We are grateful to the British Technology Group plc for generous financial support. SAC receives financial support from the Scottish Office Agriculture and Fisheries Department.

JF950772S

[⊗] Abstract published in *Advance ACS Abstracts,* September 1, 1996.