Synthesis and Antifungal Activity of Novel Bisdithiocarbamate Derivatives of Carbohydrates against *Fusarium oxysporum* f. sp. *lini*

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Novel carbamic esters possessing a carbohydrate moiety derived from glycerol or D-glucose with two *N*,*N*-diethyldithiocarbamoyl groups and a series of bisdithiocarbamic esters having a ketone or an alkyl ester have been synthesized. The in vitro activity of these new compounds was evaluated against *Fusarium oxysporum* f. sp. *lini*. Some of the compounds [bis[1,3-*S*-(*N*,*N*-diethyldithiocarbamoyl)]-1,3-dideoxyglycerol) and diethyl *N*,*N*′-(1,3-dideoxyglycer-1,3-diyl)bis(dithiocarbamate)] were more active for inhibiting vegetative mycelium growth than, respectively, the commercial *N*,*N*-diethyldithiocarbamic acid sodium salt and Maneb. The structure activity of these new compounds is discussed.

Keywords: *Bis(1,3-dithiocarbamoyl)-1,3-dideoxyglycerol; flax; in vitro antifungal activity; Fusarium oxysporum*

Dithiocarbamates are the most heavily used organic fungicides in terms of tonnage (Corbaz,1990). They are widely used for the treatment of soil, seeds, and foliar and postharvest diseases affecting several types of crops. Little resistance to these polyvalent fungicides has been observed due to their multisite mode of action (Lepoivre, 1989). Nevertheless, a problem associated with such salts is the possibility of their containing or releasing trace toxic elements or compounds (Woodrow et al., 1995; Buzasi-Gyorfy et al., 1992). They are commonly separated into two classes: dialkyldithiocarbamates including thirame sulfide such as Ziram (zinc dimethyldithiocarbamate) or *N*,*N*-diethyldithiocarbamic acid sodium salt (**I**) and alkylenebis(dithiocarbamates) such as Maneb **II**, manganese ethylenebis(dithiocarbamate)] and Nabam [disodium ethylenebis(dithiocarbamate)] (Thorn et al., 1962; Lukens, 1971).

The derivatization of known pesticides to produce propesticides, having, for example, greater residual effectiveness, continues to be a fruitful area of development (Fahmy et al., 1981; Ziegler et al., 1975). Another strategy has been to derivatize carbamates with molecules such as carbohydrates, amino acids, plant hormones, and organic acids. These molecules can have relatively high polarity and are phloem mobile. Such derivatives have been shown to increase plant systemic activity (Jojima et al., 1983). By a similar approach, we have synthesized a series of novel carbamic esters possessing a carbohydrate moiety derivative of glycerol or D-glucose, which are not associated with metal ions. The nonionic linkage between the thiocarbamoyl group and the saccharidic moiety could lead to a delayed and an extended effect. According to previous works in our laboratory (Len et al., 1996, 1997), we developed a series of esters, which associated one saccharidic moiety and two dithiocarbamoyl groups. The in vitro activities of these new compounds were evaluated against *Fusarium oxysporum* f. sp. *lini*, a major pathogenic fungus of flax (*Linum usitatissinum* L.). This soil pathogen induces widespread and very destructive vascular wilts, causing severe losses in flax crops (up to 60% of the crop). No effective chemical control agent for fusarial wilt is at the present time available. Control methods of *Fusarium* disease depends only on cultural preventive measures. Moreover, the use of resistant varieties of flax is the only effective means of controlling the disease in fields and masks the real importance of this disease. Developing chemical control agents for fusarial wilt would be very beneficial (Agrios, 1997; *La Culture du Lin Ciber*, 1999).

In the present work, we have synthesized a series of novel carbamic esters possessing one carbohydrate moiety derivative of glycerol or D-glucose with two *N*,*N*-diethyldithiocarbamoyl groups and a series of bisdithiocarbamic esters having a ketone or an alkyl ester.

The aim of this work was to compare the benefits of the carbohydrate structure and the alkyl ester and the activity between these new carbamate pesticides and both the commercial *N*,*N*-diethyldithiocarbamic acid sodium salt (**I**) and Maneb (**II**) against *F. oxysporum* f. sp. *lini*, a major pathogenic fungus of flax (*L. usitatissinum* L.).

MATERIALS AND METHODS

General. Melting points were measured with an electrothermal melting point apparatus and were uncorrected. ^{1}H (at 300.13 MHz) and ^{13}C (at 75.47 MHz) NMR spectra were recorded on a Bruker AM 300 spectrometer. Deuteriochloroform (99.8% atom enriched, Aldrich) was used as NMR solvent throughout unless otherwise stated. ^{1}H and ^{13}C NMR chemical shifts were reported in δ values based on the internal reference tetramethylsilane. Column chromatography was carried out

10.1021/jf0003698 CCC: $19.00
Published on Web 10/06/2000

with a Matrex silica gel 60 Å (70−230 mesh, Merck). Solvents and reagents were obtained from a commercial source (Aldrich).

Synthesis of Bis[1,3-*S*-(*N*,*N*-diethyldithiocarbamoyl)]-1,3-dideoxyglycerol (1). The synthesis of **1** was realized as described previously (Len et al., 1996).

Synthesis of 3,6-Dideoxybis[3,6-*S*-(*N*,*N*-diethyldithiocarbamoyl)]-1,2-*O*-isopropylidene-α-D-glucofuranose (2). 3-Deoxy-3-iodo-1,2:5,6-di-*O*-isopropylidene-α-D-allofuranose (7.0 g, 18.9 mmol) (Garegg et al., 1980) was reacted with 0.6 M HCl in water/dioxane (7:93, 65 mL) at 30 °C during 30 min. After the addition of sodium hydrogen carbonate (pH 7), the mixture was stirred for 10 min and filtered, and the solvent was removed under reduced pressure. The crude product was purified on a silica gel column eluted with hexane/acetone (1:1) to give 4.0 g (64% yield) of 3-deoxy-3-iodo-1,2-*O*-isopropylidene-α-D-allofuranose: mp 110−111 °C; $[\alpha]^{22}_D$ +113° (*c* 1.2, $CHCl_3$); ^{1}H NMR δ 5.76 (d, 1H, $J_{1,2}$ = 3.6 Hz, H-1), 4.57 (t, 1H, $J_{2,3}$ = 4.2 Hz, H-2), 4.25 (dd, 1H, $J_{4,5}$ = 2.9 Hz, H-4), 4.00 (m, 1H, $J_{5,6}$ = 7.2 Hz, H-5), 3.88 (dd, 1H, $J_{3,4}$ = 10.2 Hz, H-3), 3.83 (dd, 1H, $J_{6,6'}$ = 11.5 Hz, H-6), 3.68 (dd, 1H, $J_{5,6'}$ = 3.9 Hz, H-6′), 1.45, 1.27 [s, 6H, C(CH_3)$_2$]; ^{13}C NMR δ 111.8 [*C*(CH_3)$_2$], 103.0 (C-1), 83.5 (C-2), 81.5 (C-4), 71.5 (C-5), 63.4 (C-6), 26.5 (2C, C(*C*H_3)$_2$), 18.7 (C-3).

p-Toluenesulfonyl chloride (4.0 g, 21.2 mmol) dissolved in toluene (27 mL) was slowly added to a stirred pyridine (27 mL) solution of 3-deoxy-3-iodo-1,2-*O*-isopropylidene-α-D-allofuranose (7.0 g, 21.2 mmol) at 0 °C. After 48 h at 4 °C, crushed ice and aqueous HCl (9:1) (50 mL) were added to the mixture and the two phases separated. The aqueous phase was extracted with toluene (2 × 25 mL); the organic phases were pooled, dried (Na_2SO_4), and concentrated. The crude product was purified on a silica gel column eluted with hexane/acetone (4:1) to give 8.6 g (84% yield) of 3-deoxy-3-iodo-1,2-*O*-isopropylidene-6-*O*-tosyl-α-D-allofuranose: $[\alpha]^{22}_D$ +233° (*c* 1.2, $CHCl_3$); ^{1}H NMR δ 7.75−7.30 (4H, $J_{o,m}$ = 8.3 Hz, tosyl), 5.71 (d, 1H, $J_{1,2}$ = 3.5 Hz, H-1), 4.53 (t, 1H, $J_{2,3}$ = 4.0 Hz, H-2), 4.15 (dd, 1H, H-4), 4.15 (m, 1H, H-5), 4.15 (dd, 1H, H-3), 4.15 (dd, 1H, $J_{6,6'}$ = 10.0 Hz, H-6), 3.68 (dd, 1H, $J_{5,6'}$ = 4.0 Hz, H-6′), 1.48, 1.30 [s, 6H, C(CH_3)$_2$]; ^{13}C NMR δ 144.9 (C_{ipso}), 132.6 (C_{para}), 129.9 (C_{ortho}), 127.9 (C_{meta}), 111.9 (*C*(CH_3)$_2$), 103.0 (C-1), 82.6 (C-2), 81.5 (C-4), 69.4 (C-5), 70.7 (C-6), 26.6, 26.5 (2C, C(*C*H_3)$_2$), 18.1 (C-3).

N,*N*-Diethyldithiocarbamic acid lithium salt (1.9 g, 12.4 mmol) was added to a stirred solution of 3-deoxy-3-iodo-1,2-*O*-isopropylidene-6-*O*-tosyl-α-D-allofuranose (1.5 g, 3.1 mmol) in hexamethylphosphoramide/toluene (1:1) (5 mL). Upon completion of the addition, the reaction mixture was stirred at 110 °C for 24 h. The mixture was filtered and the solvent removed by distillation under reduced pressure to yield a viscous oil. This oily residue was treated with hexane/diethyl ether (1:1), washed twice with water, and dried (Na_2SO_4). The crude product was purified on a silica gel column eluted with hexane/acetone (9:1) to give 0.9 g (60% yield) of **2**: $[\alpha]^{22}_D$ +48° (*c* 1.1, $CHCl_3$); ^{1}H NMR δ 5.71 (d, 1H, $J_{1,2}$ = 3.9 Hz, H-1), 4.67 (d, 1H, $J_{2,3}$ = 0, H-2), 4.35 (dd, 1H, $J_{3,4}$ = 3.6 Hz, H-4), 4.05 (m, 1H, $J_{4,5}$ = 8.8 Hz, H-5), 4.69 (d, 1H, H-3), 3.86 (dd, 1H, $J_{6,6'}$ = 14.4 Hz, H-6), 3.46 (dd, 1H, $J_{5,6'}$ = 7.1 Hz, H-6′), 1.46, 1.22 [s, 6H, C(CH_3)$_2$]; ^{13}C NMR δ 196.6, 192.6 (2C, CS), 111.2 [*C*(CH_3)$_2$], 104.5 (C-1), 86.6 (C-2), 80.4 (C-4), 69.9 (C-5), 57.3 (C-3), 50.0−47.1 (4C, *C*H_2CH$_3$), 40.6 (C-6), 26.6,26.4 [2C, C(*C*H_3)$_2$], 12.5, 11.5 (4C, CH_2*C*H_3). Anal. Calcd for $C_{19}H_{34}O_4S_4N_2$ (482.75): C, 47.27; H, 7.10; S, 26.57; N, 5.80. Found: C, 47.21; H, 7.02; S, 27.02; N, 5.82.

Synthesis 3,6-Dideoxybis[3,6-*S*-(*N*,*N*-diethyldithiocarbamoyl)]-D-glucopyranose (3). 2 (2.0 g, 4.1 mmol) was reacted with a 0.6 M HCl in water/dioxane (1:9) (200 mL) at 60 °C for 2 h. After the addition of sodium hydrogen carbonate (pH 6), the mixture was stirred for 10 min and filtered, and the solvent was removed under reduced pressure. The crude product was purified on a silica gel column eluted with hexane/acetone (1:1) to give 0.9 g (50% yield) of **3**: $[\alpha]^{22}_D$ −38° (*c* 1.5, CH_3OH); ^{13}C NMR, α form δ 195.9, 194.3 (2C, CS), 91.7 (C-1), 71.2 (C-2), 70.9 (C-5), 70.7 (C-4), 57.3 (C-3), 49.3−46.4 (4C, *C*H_2CH$_3$), 40.5 (C-6), 12.3, 11.4 (4C, CH_2*C*H_3); β form δ 195.2, 194.2 (2C, CS), 98.4 (C-1), 72.3 (C-2), 71.8 (C-4), 69.5 (C-5), 60.8 (C-3), 49.3−46.4 (4C, *C*H_2CH$_3$), 40.5 (C-6), 12.3, 11.4 (4C, CH_2*C*H_3). Anal. Calcd for $C_{16}H_{30}O_4S_4N_2$ (442.68): C, 43.41; H, 6.83; S, 28.97; N, 6.33. Found: C, 43.65; H, 6.95; S, 29.30; N, 6.24.

Synthesis of 1-*S*-(*N*,*N*-Diethyldithiocarbamoyl)-DL-glycerol (4). The synthesis of **4** was realized as described previously (Len et al., 1996).

Synthesis of 2-*O*-Acetyl-1,3-dideoxy-1,3-di-*S*-(*N*,*N*-diethyldithiocarbamoyl)glycerol (5). 1 (10.0 g, 28.2 mmol) was dissolved in acetic anhydride/triethylamine (3:2) (100 mL) at room temperature for 2 h. After the addition of MeOH, the solvent was removed under reduced pressure. The crude product was purified on a silica gel column eluted with hexane/acetone (98:2) to give 8.8 g (79% yield) of **5**: ^{1}H NMR δ 5.31 (m, 1 H, H-2), 3.94 (dt, 4H, $J_{1'a,1'b}$ = 13.7 Hz, C*H*$_2$CH$_3$), 3.83 (m, 2H, $J_{1a,1b}$ = $J_{3a,3b}$ = 14.3 Hz, $J_{1a,2}$ = $J_{2,3a}$ = 4.7 Hz, H-1a, H-3a), 3.69 (dt, 4H, $J_{1''a,1''b}$ = 13.6 Hz, C*H*$_2$CH$_3$), 3.53 (dd, 2H, $J_{1b,2}$ = $J_{2,3b}$ = 6.9 Hz, H-1b, H-3b), 1.21 (m, 12H, $J_{1',2'}$ = 6.8 Hz, $J_{1'',2''}$ = 6.6 Hz, CH$_2$C*H*$_3$); ^{13}C NMR δ 194.1 (2C, CS), 169.8 (CO), 70.6 (C-2), 49.8, 46.7 (*C*H_2CH$_3$), 39.7 (C-1,C-3), 21.0 (CH_3), 12.5,11.5 (4C, CH_2*C*H_3).

Synthesis of 2-*O*-Butanoyl-1,3-dideoxy-1,3-di-*S*-(*N*,*N*-diethyldithiocarbamoyl)glycerol (6). Butanoyl chloride (1.5 g, 14.1 mmol) was added dropwise in a stirring solution of **1** (5.0 g, 14.1 mmol) and triethylamine (2.2 mL, 15.5 mmol) in toluene (40 mL) at room temperature. After 4.5 h, the mixture was filtered and the solvent was removed under reduced pressure. The crude product was purified on a silica gel column eluted with hexane/acetone (98:2) to give 3.8 g (64% yield) of **6**: ^{1}H NMR δ 5.33 (m, 1 H, H-2), 3.96 (dt, 4H, $J_{1'a,1'b}$ = 13.5 Hz, C*H*$_2$CH$_3$), 3.84 (m, 2H, $J_{1a,1b}$ = $J_{3a,3b}$ = 14.1 Hz, $J_{1a,2}$ = $J_{2,3a}$ = 4.7 Hz, H-1a, H-3a), 3.70 (dt, 4H, $J_{1''a,1''b}$ = 13.9 Hz, C*H*$_2$CH$_3$), 3.55 (dd, 2H, $J_{1b,2}$ = $J_{2,3b}$ = 7.1 Hz, H-1b, H-3b), 2.23 (t, 2H, $J_{\alpha,\beta}$ = 7.4 Hz, H-α), 1.60 (m, 2H, $J_{\beta,\gamma}$ = 7.4 Hz, H-*β*), 1.22 (m, 12H, $J_{1',2'}$ = 6.7 Hz, $J_{1'',2''}$ = 6.8 Hz, CH$_2$C*H*$_3$), 0.89 (t, 3H, $J_{\gamma,\omega}$ = 7.4 Hz, H-*γ*); ^{13}C NMR δ 194.3 (2C, CS), 172.4 (CO), 70.6 (C-2), 49.8, 46.7 (*C*H_2CH$_3$), 40.0 (C-1,C-3), 38.2 (C-α), 18.3 (C-*β*), 13.6 (C-*γ*), 12.5, 11.5 (4C, CH_2*C*H_3).

Synthesis of 2-*O*-Octanoyl-1,3-dideoxy-1,3-di-*S*-(*N*,*N*-diethyldithiocarbamoyl)glycerol (7). Likewise, octanoyl chloride (2.3 g, 14.1 mmol), **1** (5.0 g, 14.1 mmol), and triethylamine (2.2 mL, 15.5 mmol) gave, after 5 h, 5.1 g (75% yield) of **7**: ^{1}H NMR δ 5.32 (m, 1 H, H-2), 3.96 (dt, 4H, $J_{1'a,1'b}$ = 13.5 Hz, C*H*$_2$CH$_3$), 3.84 (m, 2H, $J_{1a,1b}$ = $J_{3a,3b}$ = 14.2 Hz, $J_{1a,2}$ = $J_{2,3a}$ = 4.7 Hz, H-1a, H-3a), 3.70 (dt, 4H, $J_{1''a,1''b}$ = 13.9 Hz, C*H*$_2$CH$_3$), 3.55 (dd, 2H, $J_{1b,2}$ = $J_{2,3b}$ = 7.0 Hz, H-1b, H-3b), 2.24 (dd, 2H, $J_{\alpha,\beta}$ = 7.5 Hz, H-α), 1.23 (m, 12H, $J_{1',2'}$ = 6.7 Hz, $J_{1'',2''}$ = 6.8 Hz, CH$_2$C*H*$_3$), 1.56−1.22 (m, 2H, H-*β*, H-*ξ*), 0.82 (t, 3H, $J_{\xi,\omega}$ = 5.3 Hz, H-*ω*); ^{13}C NMR δ 194.3 (2C, CS), 172.6 (CO), 70.6 (C-2), 49.8, 46.7 (*C*H_2CH$_3$), 40.0 (C-1,C-3), 34.3 (C-α), 31.6 (C-*β*), 29.0 (C-*γ*), 28.9 (C-*δ*), 24.9 (C-*ϵ*), 22.5 (C-*ζ*), 14.0 (C-*ω*), 12.5, 11.5 (4C, CH_2*C*H_3).

Synthesis of 1,3-Dideoxy-1,3-di-*S*-(*N*,*N*-diethyldithiocarbamoyl)-2-oxoglycerol (8). A solution of 1,3-dichloropropanone (10.0 g, 78.6 mmol) and *N*,*N*-diethyldithiocarbamic acid lithium salt (36.6 g, 236 mmol) in acetone (200 mL) was stirred at reflux for 3 h. The solvent was removed under reduced pressure, and the crude product was extracted twice with CH_2Cl_2/water (3:1) (50 mL). The organic phases were washed twice with water and dried (Na_2SO_4). The crude product was purified on a silica gel column eluted with hexane/acetone (9:1) to give 21.8 g (78% yield) of **8**: mp 75−78 °C; ^{1}H NMR δ 4.37 (s, 4H, H-1a, H-1b, H-3a, H-3b), 3.93 (dt, 4H, $J_{1'a,1'b}$ = 13.5 Hz, C*H*$_2$CH$_3$), 3.71 (dt, 4H, $J_{1''a,1''b}$ = 13.9 Hz, C*H*$_2$CH$_3$), 1.25 (t, 6H, *J* = 6.7 Hz, CH$_2$C*H*$_3$), 1.18 (t, 6H, *J* = 6.7 Hz, CH$_2$C*H*$_3$); ^{13}C NMR δ 198.4 (CO), 194.0 (2C, CS), 49.9 (C-3), 47.1 (C-1), 45.8 (4C, *C*H_2CH$_3$), 12.5, 11.4 (4C, CH_2*C*H_3).

Synthesis of Diethyl *N*,*N*′-(1,3-Dideoxyglycer-1,3-diyl)-bis(dithiocarbamate) (9). A solution of 1,3-dichloro-1,3-dideoxyglycerol (15.0 g, 116.3 mmol) and NaN_3 (30.2 g, 465.2 mmol) in *N*,*N*-dimethylformamide (300 mL) was stirred at reflux for 1 h. The solvent was removed under reduced pressure, and a solution of diethyl ether/water (1:1) (200 mL) was added. The aqueous phase was extracted with diethyl

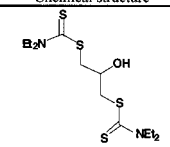
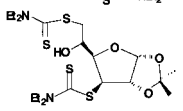
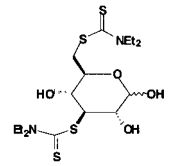
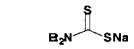
ether (50 mL), and the organic phases were washed twice with water and dried (Na_2SO_4). The solvent was removed under reduced pressure to give 17.4 g (95% yield) of 1,3-diazido-1,3-dideoxyglycerol: ^{1}H NMR δ 4.47 (s, OH), 3.82 (m, 1H, $J_{1,2} = J_{2,3} = 5.4$ Hz, H-2), 3.24 (d, 4H, H-1, H-3); ^{13}C NMR δ 69.4 (C-2), 53.6 (C-1,C-3). Triphenylphosphine (71.4 g, 271.9 mmol) was added carefully to a stirred solution of 1,3-diazido-1,3-dideoxyglycerol (16.1 g, 113.3 mmol) in THF (200 mL). After 3 h, water was introduced (10 mL) and the mixture was refluxed for 1.5 h. The solvent was removed under reduced pressure, and the crude product was boiled in 160 mL of toluene/water (1:1) for 15 min. The aqueous phase was evaporated under reduced pressure to give 6.4 g (63% yield) of 1,3-diamino-1,3-dideoxyglycerol: ^{13}C NMR δ 76.9 (C-2), 46.5 (C-1,C-3). Carbon disulfide (3.2 mL, 53.4 mmol) and triethylamine (7.4 mL, 53.4 mmol) were successively added dropwise to a stirred solution of 1,3-diamino-1,3-dideoxyglycerol (2.0 g, 22.2 mmol) in methanol/dioxane (8:1) (45 mL) at room temperature. After 1.0 h, ethyl iodide (4.23 mL, 53.3 mmol) was added and the solution stirred for 1.5 h. The solvent was removed under reduced pressure, and the crude product was purified on a silica gel column to give 10.5 g (65% yield) of **9**: ^{1}H δ 8.02 (bs, 1H, N−H), 3.94 (dd, 2H, $J_{1a,2} = J_{2,3a} = 4.8$ Hz, $J_{1a,1b} = J_{3a,3b} = 9.3$ Hz, H-1a, H-3a), 3.83 (dd, 2H, $J_{1b,2} = J_{2,3b} = 4.3$ Hz, H-1b, H-3b), 3.20 (q, 4H, $J = 7.3$ Hz, SCH_2), 1.29 (t, 6H, CH_3); ^{13}C NMR δ 200.5 (CS), 68.8 (C-2), 48.9 (C-1,C-3), 29.8 (CH_2), 14.0 (CH_3).

Stock Cultures. All of the experiments were carried out with the fungus *Fusarium oxysporum* f. sp. *lini*. This strain was obtained from the Institut National de la Recherche Agronomique (INRA Dijon, France) and was maintained on malt extract agar (MEA) slants at 22 °C with a 12 h photoperiod provided by fluorescent lamps.

Radial Fungal Growth Assays. Growth inhibition tests were conducted in vitro on MEA medium using *N*,*N*-dimethylformamide as solvent. Each fungicide solution was added to sterile molten MEA (50 °C) to provide a final concentration of 0.58 mM equivalent of dithiocarbamoyl group (molar concentration corresponding to 100 ppm of the reference *N*,*N*-diethyldithiocarbamic acid sodium salt), homogenized, and then immediately poured into 9-cm diameter polystyrene Petri dishes (20 mL per dish). This allows us to compare different compounds, which possess either one or two carbamoyl groups. The final concentration of *N*,*N*-dimethylformamide in the medium did not exceed 1% (v/v). Each dish was inoculated with a 6 mm diameter mycelial plug taken from the margin of 4-day-old *F. oxysporum* cultures on MEA. Cultures were incubated at 20 °C with a 12 h photoperiod. Growth was estimated by measuring every day the diameter of the colonies over a period of 6 days. Growth inhibition was calculated from the data of two runs of the experiment. Three replicates per treatment and per experiment were performed. Statistical analysis was performed by a two-sample *t*-test comparing treated mycelium with reference culture (at 99% confidence intervals).

Microscope Observations. The effects of these compounds on fungal morphology were also tested in Fayret liquid medium (Fayret, 1975). Each fungicide dissolved in *N*,*N*-dimethylformamide was added to 20 mL of Fayret liquid medium in 50 mL tubes to provide a final concentration of 0.58 mM equivalent of dithiocarbamoyl group. A suspension of spores was prepared by washing a 10-day-old culture of *F. oxysporum* (100 mL of MEA in a Roux flask) with 20 mL of sterile distilled water. The fragments of mycelium were removed from the spore suspension by filtration through sterile glass wool. The spore suspension was estimated using a hemocytometer. Inoculation was performed by adding a few milliliters of spore suspension in order to give a final concentration of 10^4 spores mL^{-1} of culture medium. Tubes were incubated at 20 °C on an alternative shaker (90 tpm) for 7 days with a 12 h photoperiod. The evaluation of the morphology of both untreated and treated fungi was checked using a light Olympus BX40 microscope.

Table 1. Percent Growth Inhibition of *F. oxysporum* f. sp. *lini* Caused by the Bis(dithiocarbamates) 1−3 and I

compd	Chemical structure	Inhibition (%) [a]	Statistical test [b]
1		30	S
2		17	NS
3		18	NS
I		18	Reference

[a] Growth inhibition on MEA medium supplemented with fungicide at a concentration of 0.58 mM equivalent of dithiocarbamoyl group after 6 days of culture. [b] Mean of three replicates. Statistical analysis was performed by two-sample *t*-test comparing treated culture with reference culture ($P = 0.01$). S, significantly different; NS, not significantly different.

RESULTS AND DISCUSSION

Influence of Carbohydrate Moiety on the Efficacy of the New Molecules. In the first set of experiments, quantitative analysis of the structure−activity relationships of the bisdithiocarbamates **1**−**3** having glycerol and D-glucose carbohydrate moieties were examined to determine the best natural vector.

The growth inhibitory effect of the derivatives **1**−**3** was tested in vitro on MEA plates at 100 ppm against *F. oxysporum* and compared with that of the commercial sodium *N*,*N*-diethyldithiocarbamic acid sodium salt (**I**) as a reference. All of the inhibition data were normalized as percentages of growth inhibition of *F. oxysporum* compared to the control plates using only *N*,*N*-dimethylformamide as solvent (Table 1). In unpublished work, we observed that this solvent well solubilized the bisdithiocarbamates, which are weakly soluble in most other common solvents. Commercial dithiocarbamates compounds are usually used to inhibit main flax fungi during either seedborne pathogenic infections or cultures (Champion, 1997; *La Culture du Lin Fiber*, 1999). For this reason, these fungicides were chosen for this study on the basis of the potential economical advantage of successful treatments against *F. oxysporum* f. sp. *lini*.

The nature of a carbohydrate moiety derivative of glycerol or D-glucose in new compounds **1**−**3** was significant. Among the molecules tested, only compound **1** was effective in inhibiting the mycelial growth of *F. oxysporum* f. sp. *lini* at the tested concentration on MEA plates, resulting in a 30% growth inhibition. Only a slight growth inhibition was observed for compounds **2** (17%) and **3** (18%) at the same concentration used. All of these compounds were as efficient as the reference molecule **I**.

As indicated by light microscope examination, untreated spores of *F. oxysporum* (control) germinated very quickly, before 24 h of incubation, and then the mycelium had a normal aspect, with regular septation. Sporulation occurred after 48 h of incubation. The reference molecule **I**, which presents a high polarity due to ionic charge, exhibited the best activity in liquid

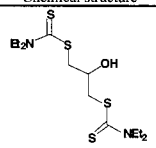
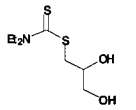
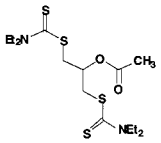
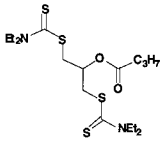
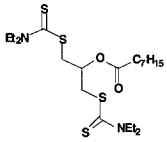
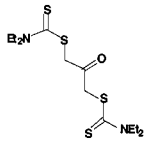
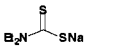
medium, inhibiting totally spore germination of *Fusarium*. During germination, swelling of spores involves an important hydratation step (Griffin, 1994) and consequently quickly allows a high uptake of carbamoyl group in *Fusarium* spores in the presence of the reference molecule **I**. In comparison, compounds **1**−**3**, which are less polar, induced a delay of spore germination. The growing mycelium showed severe morphological alterations. These alterations resulted in shorter hyphal segments and cell swellings. With compound **2**, the size and the shape of fungal cells were similar to those observed in the control, although the germination of spores was delayed. Hyphae alterations observed with compounds **1** and **3** seemed to indicate that both compounds were absorbed by mycelium. These results were confirmed by vegetative growth inhibition observed in the plate test, especially for compound **1**, which exhibited the best antifungal mycelium activity (30%). According to this result, compound **1** could be used either in preventive or in curative treatments by inhibiting vegetative mycelial growth after infection has been established on plants. Due to its efficacy, compound **1**, differing only from the other ones by the glycerol vector, should be promising. For this reason, the glycerol vector was selected for further screening.

Influence of Glycerol C-2 Functionalizations. In a second set of experiments, a quantitative analysis of structure−activity relationships of the analogue series of the basic chemical structure **1** has been conducted to design new fungicides and try to understand their mechanism of action. The antifungal activities of molecules **5**−**8** were compared to that of the commercial dialkyldithiocarbamate reference **I**. In comparison, compound **4** having one graft of carbamoyl group on glycerol was also tested against *F. oxysporum*. The results of percent growth inhibition of *F. oxysporum* caused by these compounds are presented in Table 2.

In this second experiment, compound **1**, having two carbamoyl groups on the glycerol moiety, exhibited the best antifungal activity (30%) on MEA plates. In comparison, compound **4** with one carbamate on the glycerol had a lower activity against vegetative mycelium growth and induced normal spore germination and no alteration of hyphae. This result confirmed that the presence of two carbamoyl groups on glycerol is necessary for enhancing antifungal efficacy, as observed in previous in vitro studies conducted with other fungal pathogens (Len et al., 1997).

We also examined the effect of compound **8**, which differed from the parent **1** by the presence of a ketone instead a hydroxyl group on the second position of the glycerol moiety. This compound, which had a similarly low molecular mass as **1** (respectively, 352.58 and 354.37 g mol^{-1}), induced neither delay of spore germination nor hyphal alteration in liquid culture. Moreover, this biscarbamate **8** induced a slight inhibition of mycelial growth (12%) on MEA plates. These results seemed to indicate the important part played by the hydroxyl function on the second position of the glycerol moiety, which seemed to be needed for compound efficacy. The 2-OH group might optimize the timing of liberation of the carbamic anion, which is the active moiety in such dialkyl bis(dithiocarbamates). Effectiveness seemed to depend not only on the quantity of functional groups released but also on the rate of liberation. The following results obtained with the other three molecules **5**−**7** might be consistent with such a hypothesis. These compounds differed from the basic chemical structure **1** by the presence of radical substituents on the second position of the glycerol moiety (i.e., acetyl, butanoyl, and octanoyl, respectively). Although these compounds **5**−**7** induced hyphal swellings and shortening of hyphal cells in liquid cultures similar to those observed with compound **1**, they induced a slight growth inhibition of *F. oxysporum* when compared to that observed with the parent **1** at 100 ppm on MEA plates. Moreover, no delay of spore germination was observed in liquid cultures with these compounds **5**−**7**. These results showed that the alkyl esters in the 2-OH of the glycerol moiety chains resulted in a decrease of the efficacy of the compounds. Adding longer aliphatic radicals increased the molecular weight, probably rendering this molecule less easily absorbable by fungal cells. With such compounds, the liberation of the active moiety needs a supplementary metabolic step, which is the hydrolysis of alkyl radicals. Addition of radical substituents probably delays the liberation of the active moiety and therefore reduces the efficacy of the compounds.

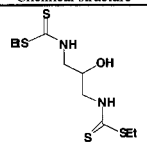
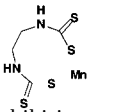
Table 2. Percent Growth Inhibition of *F. oxysporum* f. sp. *lini* Caused by the Dithiocarbamates 1, 4−8, and I

compd	Chemical structure	Inhibition (%) [a]	Statistical test [b]
1		30	S
4		10	NS
5		12	NS
6		12	NS
7		7	NS
8		12	NS
I		18	Reference

[a] Growth inhibition on MEA medium supplemented with fungicide at a concentration of 0.58 mM equivalent of dithiocarbamoyl group after 6 days of culture. [b] Mean of three replicates. Statistical analysis was performed by two-sample *t*-test comparing treated culture with reference culture ($P = 0.01$). S, significantly different; NS, not significantly different.

Versatility of the Glycerol Moiety Used as a Vector for the Two Classes of Dithiocarbamates. In the last set of experiments, we tried to investigate the impact of the glycerol vector on the antifungal activity of the second class of the dithiocarbamates: alkylenebisdithiocarbamates. These alkylenebisdithio-

Table 3. Percent Growth Inhibition of *F. oxysporum* f. sp. *lini* Caused by the Alkylenebis(dithiocarbamates) 9 and II

compd	Chemical structure	Inhibition (%) [a]	Statistical test [b]
9		27	S
II		12	Reference

[a] Growth inhibition on MEA medium supplemented with fungicide at a concentration of 0.58 mM equivalent of dithiocarbamoyl group after 6 days of culture. [b] Mean of three replicates. Statistical analysis was performed by two-sample *t*-test comparing treated culture with reference culture ($P = 0.01$). S, significantly different; NS, not significantly different.

carbamates, of which Zineb, Maneb, and Mancozeb are prominent members, represent an important group of fungicides widely used on fruits and in the treatments of vegetables (Kaars Sijpesteijn et al., 1977). Despite their chemical similarity, the dialkyldithiocarbamates (class 1) and alkylenebisdithiocarbamates (class 2) do not act in the same way. The fungitoxicity of this second class has been generally attributed to the capacity of these compounds to generate isothiocyanate, which reacts with sulfhydryl (thiol) groups of enzymes and metabolites in the cells (Ragsdale, 1994).

By a similar approach, we tried to improve the efficacity of such fungicides by grafting two dithiocarbamoyl groups onto the glycerol vector. The growth inhibitory effect of the ethyl bisdithiocarbamate **9** was tested in vitro on MEA plates at 100 ppm against *F. oxysporum*, according to procedure given under Materials and Methods and was compared to that of the classical fungicide (Maneb, **II**) as a reference. The results expressed as percent growth inhibition of *F. oxyposrum* are presented in Table 3. This compound showed higher activity than the commercial fungicide **II** on MEA plates at 100 ppm (respectively, 27 and 12% growth inhibition). In liquid culture, compound **9** induced a delay of spore germination and morphological alterations of *Fusarium* similar to those observed with compound **1**. In comparison, due to its high polarity, **II** induced a complete inhibition of spore germination in liquid culture, as observed with the commercial dialkyldithiocarbamate reference **I**. Two mechanisms may be involved in the enhancement of the fungitoxic action of this new compound: (1) production of more fungitoxic isothiocyanates or (2) inhibition of a larger number of potential sites of action, due to a wider repartition of the compound in fungal cell compartments.

The analysis of structure−activity of novel carbamic esters therefore confirmed that both compounds **1** [bis-[1,3-S-(*N*,*N*-diethyldithiocarbamoyl)]-1,3-dideoxyglycerol] and **9** [diethyl-*N*,*N*′-(1,3-dideoxyglycer-1,3-diyl)bis-(dithiocarbamate)] were the more active compounds for inhibiting mycelium growth of *F. oxysporum* f. sp. *lini* in in vitro cultures. These novel fungicides could therefore have a direct effect on the pathogen that had already invaded the plants and in this case act as eradicants by inhibiting growth and multiplication of the fungus. Nevertheless, complementary fundamental studies are needed to validate these results to identify potential targets of these novel compounds and to understand the real importance of the glycerol moiety. A question that arises from our studies is whether these new compounds possessing a glycerol moiety with dithiocarbamoyl groups have themselves antifungal activities or remain inefficacious until the release of the carbamoyl groups, known as the active moieties in dithiocarbamates. If so, these new compounds used as profungicides would be very promising as they might have unusual systemic activity in plants. To validate this hypothesis, the efficacy of these products needs to be tested in vivo in practical disease control.

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Received for review March 21, 2000. Revised manuscript received July 28, 2000. Accepted July 28, 2000.

JF0003698