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Bis(4-fluorophenyl)methyl(1*H*-1,2,4-triazol-1-yl-methyl)-germane, a germanium analogue of the agricultural fungicide flusilazole: synthesis and biological properties

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Abstract

Bis(4-fluorophenyl)methyl(1*H*-1,2,4-triazol-1-yl-methyl)germane (**2**), a germanium analogue of the agricultural fungicide flusilazole (**1**), has been synthesized from Cl_3GeCH_2Cl (**3**) by both a three-step and a four-step synthesis (**3** → $(p\text{-F-}C_6H_4)_2Ge(CH_2Cl)Br$ (**4**) → $(p\text{-F-}C_6H_4)_2Ge(CH_2Cl)CH_3$ (**5**) → **2**; **5** → $(p\text{-F-}C_6H_4)_2Ge(CH_2I)CH_3$ (**6**) → **2**). The fungicidal properties of **2** have been compared with those of the parent silicon compound **1** (studies on Si/Ge bioisosterism). In various test systems, the Si/Ge analogues **1** and **2** showed comparable fungicidal properties (in activity against plant pathogenic fungi: in agar plate diffusion tests and greenhouse evaluations; in activity against human pathogenic fungi: in serial dilution tests). In addition, **1** and **2** displayed comparable potencies in respect of sterol biosynthesis inhibition in *Saccharomycopsis lipolytica* and *Pyricularia oryzae*, the mode of action being primarily an inhibition of oxidative C14-demethylation.

Introduction

Flusilazole (**1**) (Scheme 1) is a highly potent silicon-based ergosterol biosynthesis-inhibiting fungicide [1–3] which has found acceptance worldwide for controlling diseases of cereals, grapes, tree fruits, oilseed rape, bananas, sugar beets and corn. Following initial registration in 1985, flusilazole is now one of the leading agricultural fungicides. We report here the synthesis and fungicidal properties of bis(4-fluorophenyl)methyl(1*H*-1,2,4-triazol-1-yl-methyl)germane (**2**) (Scheme1), a germa-

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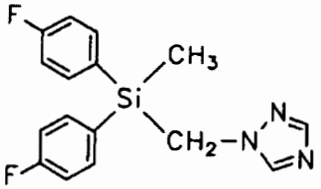

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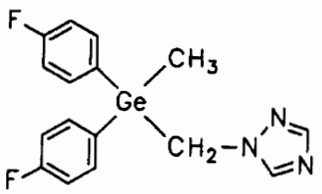


2

Scheme 1.

nium analogue of flusilazole, and a comparison is made of the biological properties of **2** with those of the silicon compound **1**.

These studies were carried out as a part of our programme on bioorganogermanium chemistry. In previous papers on this subject we reported on stereoselective microbial reductions of acetyltriorganylgermanes [4,5] and on the olfactoric properties of germa-hydratropyl acetate (a germanium analogue of the perfume hydratropyl acetate) [6]. Furthermore, radioligand-binding studies with germanium-based muscarinic antagonists were carried out [7]. Relatively little is known about the biological properties of organogermanium compounds (for the most comprehensive review on this subject, see ref. 8), whereas a large amount of information on biological effects of organosilicon compounds has been published (for a recent review on this subject, see ref. 9).

Results and discussion

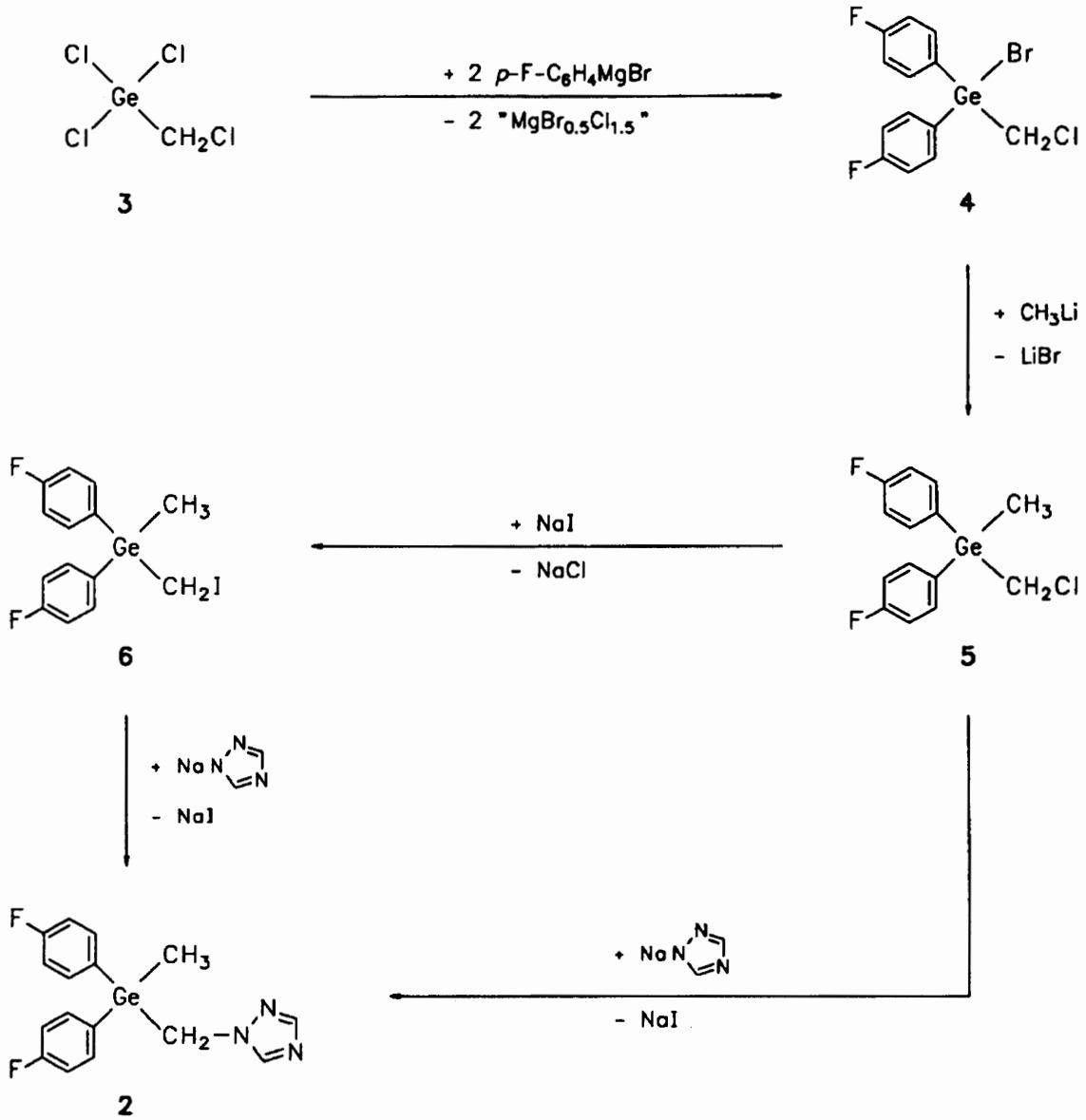
Syntheses

The germane **2** was synthesized by a three- or four-step synthesis, starting from trichloro(chloromethyl)germane (**3**) [10] and following the strategy described for the preparation of its silicon analogue **1** [1–3,11] (Scheme 2).

In the first step, bromo(chloromethyl)bis(4-fluorophenyl)germane (**4**) was prepared by reaction of **3** with (4-fluorophenyl)magnesium bromide in diethyl ether/tetrahydrofuran (yield 40%). This reaction involves the substitution of two chlorine atoms by 4-fluorophenyl groups as well as a chlorine/bromine exchange at the germanium atom. In addition, small amounts of chloro(chloromethyl)bis(4-fluorophenyl)germane ($(p\text{-F-C}_6H_4)_2Ge(CH_2Cl)Cl$) and dibromo(chloromethyl)(4-fluorophenyl)germane ($(p\text{-F-C}_6H_4)Ge(CH_2Cl)Br_2$) were obtained as by-products but could be separated from the main product **4** by distillation. In the next step, the bromogermane **4** was transformed into the corresponding methylgermane **5** by reaction with methyllithium in diethyl ether (yield 85%). Then the 1,2,4-triazol-1-yl moiety was introduced by reaction of **5** with the corresponding 1,2,4-triazole sodium salt in dimethylformamide to give **2** in 82% yield.

Alternatively, **2** was synthesized by reaction of 1,2,4-triazole sodium salt with the (iodomethyl)germane **6** (yield 90%). The latter was prepared by reaction of the corresponding (chloromethyl)germane **5** with sodium iodide in boiling acetone (yield 95%).

The germanes **4** and **5** were obtained as colourless liquids, whereas **2** and **6** were isolated as colourless (white) crystals. The structure of the germanes **2** and **4–6** was proved by elemental analysis and NMR-spectroscopic (^1H, ^{13}C) and mass-spectrometric studies.



Scheme 2.

Biological studies

The biological properties of the Si/Ge analogues **1** and **2** were compared by the following methods: (i) To test their fungicidal activity against plantpathogenic fungi, the minimal inhibition concentrations (MIC values) of the compounds were determined in an agar plate diffusion test for six selected strains of microorganisms (results in Table 1). (ii) To test their fungicidal activity against human pathogenic fungi, the MIC values of **1** and **2** were determined in a serial dilution test for five selected strains of microorganisms (results in Table 2). (iii) The fungicidal properties of the compounds were also studied under greenhouse conditions for ten different pathosystem/plant models (results in Table 3). (iv) In addition, **1** and **2** were tested for their potency with respect to sterol biosynthesis inhibition and for their mode of action in *Saccharomycopsis lipolytica* and *Pyricularia oryzae* (results in Tables 4 and 5).

As can be seen from Table 1, the Si/Ge analogues **1** and **2** showed comparable fungicidal activities against plant pathogenic fungi. This is also true for human

Table 1

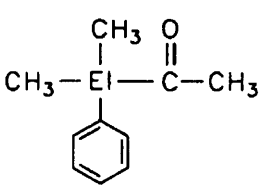
Fungicidal activity (MIC values) of the Si/Ge analogues **1** and **2** against plant pathogenic fungi

Fungus	MIC value (ppm)	
	1	**2**
Saccharomycopsis lipolytica	1.7	1.6
Pyricularia oryzae	0.9	1.0
Pseudocercosporella herpotrichoides	0.3	0.5
Fusarium culmorum	9.2	1.9
Botrytis cinerea	3.1	8.9
Pyrenophora teres	1.2	0.7

Table 2

Fungicidal activity (MIC values) of the Si/Ge analogues **1** and **2** against human pathogenic fungi

Fungus	MIC value (μg/ml)	
	1	**2**
Trichophyton mentagrophytes	1	1
Aspergillus fumigatus	2	8
Candida albicans	8	16
Candida glabrata	16	32
Candida tropicalis	32	64



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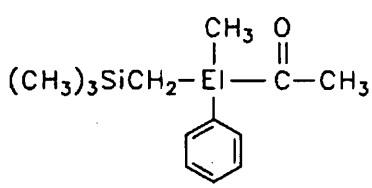
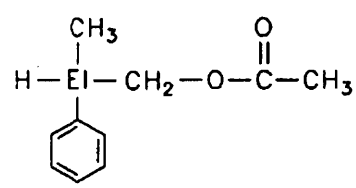
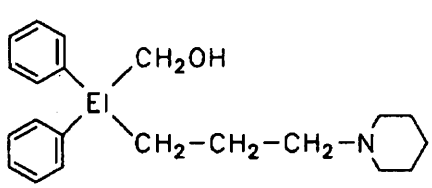
8: El = Ge



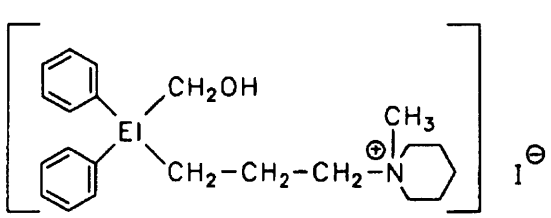
9: El = Si
10: El = Ge



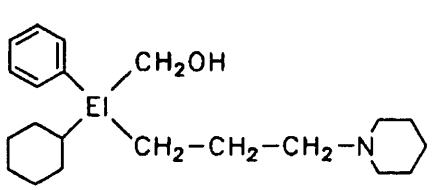
11: El = Si
12: El = Ge



13: El = Si
14: El = Ge



15: El = Si
16: El = Ge



17: El = Si
18: El = Ge

Scheme 3.

Table 3

Fungicidal properties of the Si/Ge analogues 1 and 2 under greenhouse conditions

Pathosystem (plant)	Spray conc. (ppm a.i.)	Fungicidal activity (%)		Disease severity (%) (untreated control)
		1	2	
Sphaerotheca fuliginea (cucumber)	0.1	67	71	85
Venturia inaequalis	0.4	100	100	63
(apple)	0.2	100	100	
Cercospora canescens	5	95	91	58
(mungbean)				
Septoria nodorum	10	100	100	20
(wheat)	5	37	37	
Pyrenophora teres	25	83	67	75
(barley)	10	83	67	
	5	67	67	
Cochliobolus sativus	25	83	83	75
(barley)	10	83	83	
	5	83	83	
Pseudocercosporella	100	100	100	100
herpotrichoides	50	100	0	
(wheat)	25	0	0	
Puccinia recondita	25	66	71	100
(wheat)	5	25	25	
	1	0	9	
Erysiphe graminis	25	88	100	100
(barley)	5	88	88	
	1	41	41	
	0.5	25	25	
Erysiphe graminis	5	100	100	100
(wheat)	1	100	100	
	0.5	91	88	

pathogenic fungi (Table 2). Under greenhouse conditions, **1** and **2** also displayed similar fungicidal properties (Table 3). In addition, the compounds showed comparable potencies with respect to sterol biosynthesis inhibition in *Saccharomycopsis lipolytica* and *Pyricularia oryzae* (Tables 4 and 5). The sterol distribution pattern in

Table 4

Sterol pattern in *Saccharomycopsis lipolytica* after treatment with 1 ppm of the Si/Ge analogues 1 and 2

Sterol type	Sterol (%)		
	Untreated	After treatment [a]	
		1	2
Ergosterol	100	54.4	48.2
24,25-Dihydrolanosterol	< 0.02	28.6	41.9
Lanosterol	< 0.02	12.3	7.3

[a] Values do not sum to 100% because of trace amounts of various other steriods.

Table 5

Sterol pattern in *Pyricularia oryzae* after treatment with 10 ppm of the Si/Ge analogues 1 and 2

Sterol type	Sterol (%)		
	Untreated	After treatment	
		1	2
Ergosterol	92.1	< 0.02	4.9
Δ^5-Stigmastenol	7.9	37.1	26.9
Δ^5-Ergostenol	< 0.02	10.5	8.7
$\Delta^{5,22}$-Stigmastadienol	< 0.02	< 0.02	7.1
24-Methylenedihydrolanosterol	< 0.02	52.4	52.4

these fungi revealed that the mode of action of **1** and **2** is primarily an inhibition of oxidative C14-demethylation (in this context see ref. 12).

In conclusion, the Si/Ge analogues **1** and **2** showed comparable biological properties. Similar results were obtained in a study of the interaction of other structurally analogous silicon and germanium compounds with various biological systems: (i) The acetylgermanes **8** and **10** were found to undergo an analogous (*R*)-selective microbial reduction with cells of the yeast *Trigonopsis variabilis* (DSM 70714) as observed for the corresponding acetylsilanes **7** and **9** (≡El–C(O)Me → ≡El–C(OH)HMe (El = Si, Ge); Scheme 3) [4,5]. (ii) The silane **11** and the germane **12** showed comparable olfactoric properties, indicating similar interactions of these Si/Ge analogues with the receptors of the human olfactory system [6]. (iii) The germanium compounds **14**, **16** and **18** showed affinities for rat cardiac muscarinic M2 receptors similar to those of their silicon analogues **13**, **15** and **17**; the respective Si/Ge analogues revealed comparable thermodynamic parameters (enthalpy and entropy changes) for the receptor binding [7]. (iv) Preliminary studies on the metabolic fate of **13** and **14** in the rat, after oral administration, revealed analogies in the phase-I metabolism of these Si/Ge analogues [13]. From these results and the data published in this paper, it may be concluded that there are distinct bioisosteric relationships between structurally analogous silicon and germanium compounds.

Experimental

Syntheses

All syntheses were performed in dried solvents (boiling range of the petroleum ether used: 40–65°C) under dry nitrogen. Dimethylformamide (Fluka Chemie, 40250) was purified prior to use by dynamic drying over an aluminium oxide (Merck 1077) column. 1,2,4-Triazole sodium salt (Aldrich 19,764-5) was purified prior to use by three reprecipitations from ethanol/diethyl ether and subsequent drying *in vacuo*. (The salt (20 g) was dissolved in boiling ethanol (100 ml) and after filtration of the hot solution, reprecipitated at room temperature by addition of diethyl ether (600 ml).) Melting points were determined with a Büchi 530 apparatus (oil bath) and are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded either on a Bruker AM-400 spectrometer (400.1 and 100.6 MHz, respectively) or on a Bruker AC-200 spectrometer (200.1 and 50.3 MHz, respectively). Chemical shifts

(ppm) were determined relative to internal TMS (^{1}H, δ 0) or $CDCl_3$ (^{13}C, δ 77.05). Assignment of the ^{13}C data was supported by DEPT experiments; the results of these experiments are included in the assignments. EI mass spectra (70 eV) were obtained with a Finnigan MAT 8430 mass spectrometer; the *m/z* values given (selected characteristic ions) refer to the isotopes ^{1}H, ^{12}C, ^{14}N, ^{19}F, ^{35}Cl, ^{74}Ge, ^{79}Br and ^{127}I.

Bis(4-fluorophenyl)methyl(1H-1,2,4-triazol-1-yl-methyl)germane (2). Method a: A mixture of **5** (1.88 g, 5.74 mmol) and 1,2,4-triazole sodium salt (0.55 g, 6.04 mmol) in dimethylformamide (5 ml) was stirred at 90°C for 3 h. The resulting slurry was cooled to room temperature and diluted with water (10 ml). After three extractions of the mixture with 20 ml portions of diethyl ether, the combined ethereal extracts were washed with saturated aqueous sodium chloride solution (10 ml) and then dried over anhydrous Na_2SO_4. The solvent was removed under reduced pressure and the residue purified by Kugelrohr distillation (170°C/0.1 Torr) to give 1.70 g (yield 82%) of a yellow oily liquid. Crystallization of the product from diethyl ether/petroleum ether (1:8, v/v) at −20°C yielded colourless crystals, m.p. 41–42°C.

Method b: A mixture of **6** (2.40 g, 5.73 mmol) and 1,2,4-triazole sodium salt (0.55 g, 6.04 mmol) in dimethylformamide (5 ml) was stirred at 90°C for 3 h and the resulting slurry was worked up as described under Method a; yield 1.86 g (90%).

^{1}H NMR ($CDCl_3$): δ 0.76 (s, 3H, $GeCH_3$); 4.30 (s, 2H, $GeCH_2N$); 7.05–7.1 and 7.4–7.45 (m, 8H, GeC_6H_4F); 7.81 (s, 1H, N–CH=N); 7.87 (s, 1H, N–CH=N). ^{13}C NMR ($CDCl_3$): δ −4.7 ($GeCH_3$); 39.6 ($GeCH_2N$); 115.8 (d, ^2J(CF) = 20.2 Hz, C-3/C-5, GeC_6H_4F); 130.9 (d, ^4J(CF) = 3.8 Hz, C-1, GeC_6H_4F); 135.7 (d, ^3J(CF) = 7.4 Hz, C-2/C-6, GeC_6H_4F); 143.0 (N–CH=N); 151.6 (N–CH=N); 163.9 (d, ^1J(CF) = 249.2 Hz, C-4, GeC_6H_4F). MS: *m/z* 361 (9%, M^+), 346 (22%, $M^+ - CH_3$), 293 (6%, $M^+ - C_2H_2N_3$), 279 (100%, $M^+ - C_3H_4N_3$). Anal. Found: C, 53.1; H, 4.2; N, 11.7. $C_{16}H_{15}F_2GeN_3$ (359.9) calc.: C, 53.40; H, 4.20; N, 11.68%.

Trichloro(chloromethyl)germane (3). This was made as described in ref. 10.

Bromo(chloromethyl)bis(4-fluorophenyl)germane (4). A Grignard reagent was prepared from 1-bromo-4-fluorobenzene (33.3 g, 190 mmol) and magnesium turnings (4.62 g, 190 mmol) in diethyl ether (80 ml) and was then added dropwise at room temperature during 1 h to a stirred solution of **3** (20.0 g, 87.6 mmol) in diethyl ether/petroleum ether (1.3:1, v/v) (350 ml). After 15 h stirring at room temperature and heating under reflux for 6 h, the precipitate was filtered off and washed with petroleum ether (200 ml). The filtrate was combined with the washings and the solvent removed under reduced pressure. Petroleum ether (500 ml) was added to the oily residue and the mixture kept at −20°C for 1 day, and the resulting precipitate then filtered off and washed with petroleum ether (200 ml). The solvent was removed from the combined organic solutions under reduced pressure and the residue distilled *in vacuo* (Vigreux column) to give 21.2 g of a colourless liquid, b.p. 109°C/0.001 Torr, consisting of a mixture of about 85 mol% **4**, 8 mol% chloro(chloromethyl)bis(4-fluorophenyl)germane and 7 mol% dibromo(chloromethyl)(4-fluorophenyl)germane (^{1}H NMR analysis). Repeated fractional distillation yielded 13.6 g (yield 40%) of pure (^{1}H NMR) **4**.

^{1}H NMR ($CDCl_3$): δ 3.67 (s, 2H, $GeCH_2Cl$); 7.1–7.2 and 7.6–7.7 (m, 8H, GeC_6H_4F). ^{13}C NMR ($CDCl_3$): δ 30.4 ($GeCH_2Cl$); 116.2 (d, ^2J(CF) = 20.8 Hz, C-3/C-5, GeC_6H_4F); 127.9 (d, ^4J(CF) = 3.8 Hz, C-1, GeC_6H_4F); 136.1 (d, ^3J(CF)

= 8.0 Hz, C-2/C-6, GeC_6H_4F); 164.7 (d, $^1J(CF)$ = 251.5 Hz; C-4, GeC_6H_4F). MS: *m/z* 343 (100%, $M^+ - CH_2Cl$). Anal. Found: C, 39.8; H, 2.7. $C_{13}H_{10}BrClF_2Ge$ (392.2) calc.: C, 39.82; H, 2.57%.

(Chloromethyl)bis(4-fluorophenyl)methylgermane (5). A 1.78 *M* solution of methyllithium in diethyl ether (35.5 ml, 63.2 mmol CH_3Li) was added dropwise at room temperature during 30 min to a stirred solution of **4** (20.0 g, 51.0 mmol) in diethyl ether (50 ml). After 15 h stirring at room temperature and heating under reflux for 4.5 h, the mixture was cooled to 0°C and treated with saturated aqueous NaCl solution (50 ml). The organic phase was separated and the aqueous layer extracted three times with 20 ml portions of diethyl ether. The combined organic layers were dried over anhydrous Na_2SO_4, the solvent removed under reduced pressure, and the oily residue was distilled *in vacuo* (short Vigreux column) to give 14.2 g (yield 85%) of a colourless liquid, b.p. 109°C/0.01 Torr.

^1H NMR ($CDCl_3$): δ 0.78 (s, 3H, $GeCH_3$); 3.34 (s, 2H, $GeCH_2Cl$); 7.05–7.1 and 7.4–7.5 (m, 8H, GeC_6H_4F). ^{13}C NMR ($CDCl_3$): δ −5.6 ($GeCH_3$); 29.2 ($GeCH_2Cl$); 115.7 (d, $^2J(CF)$ = 20.1 Hz, C-3/C-5, GeC_6H_4F); 131.5 (d, $^4J(CF)$ = 3.8 Hz, C-1, GeC_6H_4F); 135.8 (d, $^3J(CF)$ = 7.3 Hz, C-2/C-6, GeC_6H_4F); 163.9 (d, $^1J(CF)$ = 248.7 Hz, C-4, GeC_6H_4F). MS: *m/z* 328 (< 1%, M^+), 313 (1%, $M^+ - CH_3$), 279 (100%, $M^+ - CH_2Cl$). Anal. Found: C, 51.4; H, 4.2; Cl, 11.0. $C_{14}H_{13}ClF_2Ge$ (327.3) calc.: C, 51.38; H, 4.00; Cl, 10.83%.

Bis(4-fluorophenyl)(iodomethyl)methylgermane (6). A mixture of **5** (12.9 g, 39.4 mmol) and sodium iodide (17.7 g, 118 mmol) in acetone (100 ml) was stirred under reflux for 21 h, then allowed to cool to room temperature. The precipitate was filtered off and washed with petroleum ether (30 ml). The filtrate was combined with the washings and the solvent removed under reduced pressure. Petroleum ether (300 ml) was added to the oily residue, the mixture kept at room temperature for 1 h, the resulting precipitate filtered off, and the solvent removed under reduced pressure. Vacuum distillation (Vigreux column) of the residue yielded 15.7 g (yield 95%) of a colourless liquid, b.p. 122–130°C/0.001 Torr, which crystallized spontaneously at room temperature. Recrystallization from diethyl ether/petroleum ether (1:6, v/v) at −20°C gave white crystals, m.p. 46°C. ^1H NMR ($CDCl_3$): δ 0.79 (s, 3H, $GeCH_3$); 2.48 (s, 2H, $GeCH_2I$); 7.05–7.1 and 7.4–7.5 (m, 8H, GeC_6H_4F). ^{13}C NMR ($CDCl_3$): δ −18.6 ($GeCH_2I$); −3.9 ($GeCH_3$); 115.6 (d, $^2J(CF)$ = 20.1 Hz, C-3/C-5, GeC_6H_4F); 132.2 (d, $^4J(CF)$ = 3.8 Hz, C-1, GeC_6H_4F); 135.7 (d, $^3J(CF)$ = 7.4 Hz, C-2/C-6, GeC_6H_4F); 163.8 (d, $^1J(CF)$ = 248.7 Hz, C-4, GeC_6H_4F). MS: *m/z* 420 (1%, M^+), 279 (100%, $M^+ - CH_2I$). Anal. Found: C, 40.2; H, 3.1. $C_{14}H_{13}F_2GeI$ (418.7) calc.: C, 40.16; H, 3.13%.

Biological studies

Determination of fungicidal activities of 1 and 2 against plant pathogenic fungi (agar plate diffusion test). The fungicidal activities (MIC values) of **1** and **2** against plant pathogenic fungi were determined in an agar plate diffusion test. One hundred microlitres each of seven decreasing concentrations of aqueous solutions of **1** or **2** were applied to holes (diameter 10 mm) in a 1 mm thick agar containing mycelia suspensions of the test organisms. After incubation at 28°C for 24 h, round inhibition zones were observed around the holes as compared to the normally growing regions. The diameter of the inhibition zones (mm) *versus* log *c* (*c* = concentration of the test compounds) resulted in a linear regression, which

was extrapolated to the 10 mm hole diameter for calculation of the MIC values. The results of these studies are summarized in Table 1.

Determination of fungicidal activities of 1 and 2 against human pathogenic fungi (serial dilution test). The fungicidal activities (MIC values) of **1** and **2** against human pathogenic fungi were determined in a serial dilution test, the yeasts (*Candida albicans, Candida glabrata, Candida tropicalis*) in the medium YNB from Difco, the other fungi in Kimmig medium. In all cases 1×10^5 colony forming units per millilitre, photometrically determined, were used. The yeasts and *Aspergillus fumigatus* were incubated at 35°C, *Trichophyton mentagrophytes* at 28°C. Evaluations were carried out after 48 h for the yeasts, after 72 h for *Aspergillus fumigatus*, and after 96 h for *Trichophyton mentagrophytes*. The results of these studies are summarized in Table 2.

Determination of fungicidal activities of 1 and 2 against plant pathogenic fungi under greenhouse conditions. The fungicidal properties of **1** and **2** were studied in the greenhouse with ten different pathosystem/plant models (examples 1–10). The results of these studies are summarized in Table 3.

Example 1 (Sphaerotheca fuliginea *(cucumber) / protective):* To produce a suitable preparation of active compound, 1 part by weight of **1** or **2** was mixed with the solvent acetone (94 parts by weight) and the emulsifier alkylaryl polyglycol ether (6 parts by weight), and the concentrate was diluted with water to the desired concentration. To test for protective activity, young cucumber plants were sprayed with the various preparations until dew moist. After the spray coating had dried, the plants were inoculated with conidia of the fungus *Sphaerotheca fuliginea* and then placed in a greenhouse at a temperature of 23–24°C and a relative humidity of about 75%. Evaluation was carried out 21 days after the inoculation.

Example 2 (Venturia inaequalis *(apple) / protective):* Young apple plants were sprayed with preparations of **1** or **2** (prescriptions as in example 1) until dew moist. After the spray coating had dried, the plants were inoculated with a water suspension of conidia of the fungus *Venturia inaequalis* and placed in an incubation cabin at 20°C and 100% relative humidity for 24 h. The plants were then placed in a greenhouse at a temperature of 20°C and a relative humidity of about 70%. Evaluation was carried out 12 days after the inoculation.

Example 3 (Cercospora canescens *(mungbean) / protective):* Young mungbean plants were sprayed with preparations of **1** or **2** (prescriptions as in example 1) until dew moist. After the spray coating had dried, the plants were inoculated with a water suspension of spores of *Cercospora canescens* and were placed in a dark cabin at 22°C and 100% relative humidity for 96 h. The plants were then placed in a greenhouse at a temperature of 23°C and a relative humidity of 80%. Evaluation was carried out 20 days after the inoculation.

Example 4 (Leptosphaeria (Septoria) nodorum *(wheat) / protective):* To produce a suitable preparation of active compound, 1 part by weight of **1** or **2** was mixed with the solvent dimethylformamide (100 parts by weight) and the emulsifier alkylaryl polyglycol ether (0.25 parts by weight), and the concentrate was diluted with water to the desired concentration. To test for protective activity, young wheat plants were sprayed with the various preparations until dew moist. After the spray coating had dried, the plants were sprayed with a spore suspension of *Leptosphaeria nodorum* and placed in an incubation cabin at 20°C and 100% relative humidity for 48 h. The plants were then placed in a greenhouse at a

temperature of about 15°C and a relative humidity of about 80%. Evaluation was carried out 10 days after the inoculation.

Example 5 (Pyrenophora teres *(barley) / protective):* To test for protective activity, young barley plants were sprayed with preparations of **1** or **2** (prescriptions as in example 4) until dew moist. After the spray coating had dried, the plants were sprayed with a conidia suspension of *Pyrenophora teres* and placed in an incubation cabin at 20°C and 100% relative humidity for 48 h. The plants were then placed in a greenhouse at a temperature of about 20°C and a relative humidity of about 80%. Evaluation was carried out 7 days after the inoculation.

Example 6 (Cochliobolus sativus *(barley) / protective):* To test for protective activity, young barley plants were sprayed with preparations of **1** or **2** (prescriptions as in example 4) until dew moist. After the spray coating had dried, the plants were sprayed with a conidia suspension of *Cochliobolus sativus* and placed in an incubation cabin at 20°C and 100% relative humidity for 48 h. The plants were then placed in a greenhouse at a temperature of about 20°C and a relative humidity of about 80%. Evaluation was carried out 7 days after the inoculation.

Example 7 (Pseudocercosporella herpotrichoides *(wheat) / protective):* To test for protective activity, young wheat plants were sprayed with preparations of **1** or **2** (prescriptions as in example 4) until dew moist. After the spray coating had dried, the plants were inoculated with spores of *Pseudocercosporella herpotrichoides* at the stem basis. The plants were then placed in a greenhouse at a temperature of about 10°C and a relative humidity of about 80%. Evaluation was carried out 21 days after the inoculation.

Example 8 (Puccinia recondita *(wheat) / protective):* To test for protective activity, young wheat plants were inoculated with a spore suspension of *Puccinia recondita* in a 0.1% aqueous agar solution. After the spore suspension had dried, the plants were sprayed with preparations of **1** or **2** (prescriptions as in example 4) until dew moist and placed in an incubation cabin at 20°C and 100% relative humidity for 24 h. The plants were then placed in a greenhouse at a temperature of about 20°C and a relative humidity of about 80%. Evaluation was carried out 10 days after the inoculation.

Example 9 (Erysiphe graminis *(barley) / protective):* To test for protective activity, young barley plants were sprayed with preparations of **1** and **2** (prescriptions as in example 4) until dew moist. After the spray coating had dried, the plants were dusted with spores of *Erysiphe graminis* f. sp. *hordei* and were then placed in a greenhouse at a temperature of about 20°C and a relative humidity of about 80%. Evaluation was carried out 7 days after the inoculation.

Example 10 (Erysiphe graminis *(wheat) / protective):* To test for protective activity, young wheat plants were sprayed with preparations of **1** or **2** (prescriptions as in example 4) until dew moist. After the spray coating had dried, the plants were dusted with spores of *Erysiphe graminis* f. sp. *tritici* and then placed in a greenhouse at a temperature of about 20°C and a relative humidity of about 80%. Evaluation was carried out 7 days after the inoculation.

Mode of action studies

Compounds **1** and **2** were compared with respect to their potency in sterol biosynthesis inhibition in *Saccharomycopsis lipolytica* and in *Pyricularia oryzae*. The fermentation of the microorganisms and the isolation of the sterols were

carried out as described in refs. 14 and 15 (see also ref. 16). The results of these studies are summarized in Tables 4 and 5.

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