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AN ANTIBIOTIC FROM *TRICHODERMA KONINGII* ACTIVE AGAINST SOILBORNE PLANT PATHOGENS

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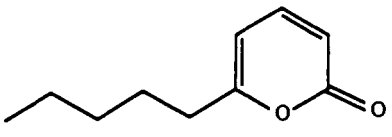
ABSTRACT.—A new antibiotic has been obtained as the major metabolite produced in pure culture by *Trichoderma koningii* isolated from soil suppressive to the saprophytic growth of the take-all fungus, *Gaeumannomyces graminis* var. *tritici*. The structure of the compound, 4,8-dihydroxy-2-(1-hydroxyheptyl)-3,4,5,6,7,8-hexahydro-2*H*-1-benzopyran-5-one [**2**], has been deduced by spectroscopic methods that also allow the relative stereochemistry (2*S**, 4*R**, 8*R**, 1′*S**) to be assigned. The compound and broth culture containing the compound inhibited the growth of the take-all fungus in vitro. The compound produced in agar culture inhibited the growth of several other soilborne plant pathogens.

In recent years considerable interest has been shown in the use of *Trichoderma* spp. as biological control agents (1). A number of species are known to produce volatile (2) and nonvolatile (3) antibiotics in vitro. Cultures of *Trichoderma koningii* Oudem. (Deuteromycotina) were isolated from soil suppressive to the saprophytic growth of *Gaeumannomyces graminis* (Sacc.) Arx and Olivier var. *tritici* Walker (*Ggt*), the causal agent of take-all of cereals (4). One isolate of *T. koningii* produced the volatile compound 6-*n*-pentyl-2*H*-pyran-2-one [**1**], which inhibited the growth in vitro of *Ggt* and several other soilborne plant pathogens (5). Similar results have been described (6) for **1** produced by two



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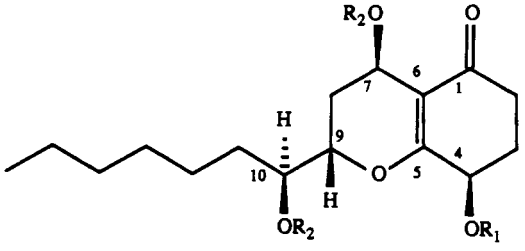

	R_1	R_2
2*	H	H
4	Ac	Ac
5	Ac	H

*Relative stereochemistry only. For convenience a non-systematic numbering is used.

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isolates of *Trichoderma harzianum*. Investigation of a second isolate of *T. koningii* has led to the isolation of a nonvolatile antibiotic as the major metabolite. The structure of this new compound, 4,8-dihydroxy-2-(1-hydroxyheptyl)-3,4,5,6,7,8-hexahydro-2*H*-1-benzopyran-5-one [**2**], has been established by spectroscopic methods. Evidence for its relative stereochemistry and details of its in vitro biological activity are presented.

RESULTS AND DISCUSSION

The filter-sterilized broth culture of the the fungus inhibited growth of *Ggt* in pure culture (Table 1). The inhibition was not as great as that achieved under the same test conditions using the broth culture of the isolate of *T. koningii* that produced **1** (5).

TABLE 1. Effect of Broth Culture of *Trichoderma koningii* on the Growth of *Gaeumannomyces graminis* var. *tritici* (*Ggt*) in Agar Culture,[a] Measured as Colony Area (mm^2). Values are means of five replicates, with SEM given in parentheses.

Broth culture	*Ggt* growth period (days at 20°)		
	2	3	4
T. koningii[b]	128 (5)	297 (22)	546 (56)
PDB[c]	397 (7)	922 (14)	1595 (13)
unamended PDA . .	340 (20)	770 (45)	1326 (83)

[a]*Ggt* grown on ⅓ strength Potato Dextrose Agar (PDA).

[b]⅓ strength Potato Dextrose Broth (PDB) culture of *T. Koningii* sterilized by passage through filter membrane with pore size 0.22 μm and 2 ml mixed with 20 ml ⅓ PDA in Petri dish.

[c]Sterile PDB mixed with PDA at same concentration.

Extraction of the culture broth with EtOAc gave a yellow oil, which by tlc analysis appeared to contain one major and several minor components. Isolation of the major compound (R_f 0.13, Si gel, EtOAc) was achieved by chromatography on alumina (activity I, neutral). Flash chromatography on silicic acid gave the major metabolite contaminated with another compound of slightly higher R_f. Bioassay of the sample of pure compound showed it to be responsible for the biological activity found in the broth culture (Table 2).

TABLE 2. Inhibition of Growth of *Gaeumannomyces graminis* var. *tritici* (*Ggt*) in vitro (Measured as Area of Colony in mm^2 on ⅓ Strength Potato Dextrose Agar) by Compound **2** Produced in Broth Culture by *Trichoderma koningii*. Values are means of three replicates with SEM given in parentheses.

Compound	Growth period of *Ggt* (days at 20°)				
	2	3	4	5	6
2[a]	32 (2)	33 (1)	63 (2)	198 (27)	549 (48)
Ethanol Control[b]	110 (3)	278 (5)	604 (18)	1033 (29)	1628 (18)
Blank	129 (9)	356 (27)	761 (21)	1324 (53)	1987 (56)

[a]Compound obtained by cc of extract of broth culture, dissolved in 1 ml of EtOH and 10 μl of solution applied directly onto the inoculum plug of *Ggt*.

[b]10 μl EtOH applied directly onto plug of *Ggt*.

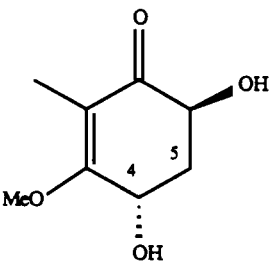
The metabolite produced in agar culture inhibited the growth in vitro of all six soilborne plant pathogens tested (Table 3). Tlc analysis (Si gel, EtOAc) of the EtOAc extract of the agar culture indicated that the compound produced in agar culture was identical to **2**.

TABLE 3. Effect of Antibiotic Produced by *Trichoderma koningii* in Agar Culture[a] on the Subsequent Growth of Several Soilborne Plant Pathogens, Measured as Colony Area (mm^2). Values are means of three replicates with SEM given in parentheses.

Pathogen	Growth period (days at 20°)	*T. koningii*	
		−	+
Gaeumannomyces graminis var. *tritici*	1	98 (9)	40 (1)
	2	537 (37)	42 (1)
	3	1152 (18)	44 (2)
Rhizoctonia solani	1	197 (1)	38 (2)
	2	929 (14)	42 (3)
	3	1662 (27)	46 (1)
Phytophthora cinnamomi	1	114 (3)	37 (2)
	2	472 (1)	38 (1)
	3	882 (10)	92 (6)
Pythium middletonii	1	593 (19)	38 (1)
	2	3011 (41)	184 (4)
	3	5076 (66)	232 (11)
Fusarium oxysporum	1	133 (5)	46 (2)
	2	475 (11)	103 (7)
	3	792 (18)	170 (22)
Bipolaris sorokiniana	1	92 (3)	44 (1)
	2	278 (15)	46 (1)
	3	450 (14)	45 (2)

[a]*T. koningii* grown on dialysis membrane overlay of ⅓ strength Potato Dextrose Agar for 4 days at 15° and then membrane and hyphae removed. Agar then inoculated with culture of pathogen.

The major metabolite **2** was obtained as an amorphous solid that gave small solvated needles on crystallization from $CHCl_3$/pentane but only a gelatinous precipitate from a number of other common solvents. Hrms of **2** showed it to have a molecular formula of $C_{16}H_{26}O_5$. The ^{13}C-nmr spectrum of **2** contained signals for three sp^2 carbons assigned to a carbonyl group (δ 198.5 s) and two olefinic carbons (δ 171.5 s, 113.9 s); thus, the compound is bicyclic. Evidence for the presence of an α,β-unsaturated cyclohexenone was obtained from the ir [ν max (CCl_4) 1655, 1620; ν max ($CHCl_3$) 1650, 1620 cm^{-1}] and uv spectra [λ max (EtOH) 260 nm, log ε 3.9].



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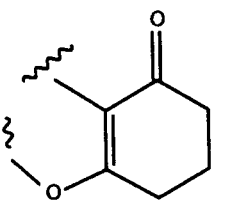


A

A β-oxy substituent was indicated by the uv absorption maximum, which did not shift on addition of base to the solution and, thus, must be part of an enol ether moiety. This contention is supported by the low field resonance (δ 171.5 for one of the two olefinic carbons of a tetrasubstituted double bond. On this basis the partial structure **A** can be considered. The similarity of the ir and uv absorption parameters exhibited by **2** and those described (7) for **3** [λ max (EtOH) 267 nm, log ε 4.14; ν (nujol) 1650, 1620 cm^{-1}], the methyl ether of a metabolite of *Aspergillus terreus*, is striking.

The nature of the three remaining oxygens as secondary hydroxyl groups was established by formation of a triacetate **4**. The ^{1}H-nmr spectrum of **4** showed a significant deshielding effect for three of the four oxymethine protons present in the spectrum of **2**, indicating that the enol oxygen is linked to a trisubstituted carbon. The chemical shifts for two of three hydroxy methine protons (δ 4.6 and 4.4) suggest that they are allylic. ^{1}H-^{1}H correlation and extensive decoupling experiments (Table 4) revealed the relative position of the oxygen atoms in **2**. Thus, the allylic hydroxymethine at C-7 (δ 4.6), apart from homoallylic coupling (J = 1 Hz) to H-4, was shown to have equal coupling (J = 4 Hz) to two methylene protons (δ 2.0 and 1.6, J = 15 Hz). These in turn showed a large (J = 12 Hz) and a small (J = 2 Hz) coupling to the oxymethine proton at δ 4.1, whose chemical shift was essentially unchanged in the spectrum of the triacetate; the proton is assigned H-9. The proton at δ 4.1 showed a coupling of 7 Hz to the third hydroxymethine (δ 3.7), which appeared as a ddd (J = 7,7,7 Hz) with two equal couplings to methylene hydrogens at δ 1.3 and 1.6. The connectivity between C-4–C-3–C-2 was similarly established. Thus, the second allylic hydroxymethine at δ 4.4 was shown to have couplings to methylene hydrogens (δ 2.0, 2.2) that were in turn coupled

TABLE 4. ^{1}H- and ^{13}C-nmr Data of the Triol **2**.[a]

Position	δ ^{13}C[b,c]	Position	δ ^{1}H	$J_{H,H}$ (Hz)
1	195.8	2a	2.3	$J_{2a,2b}$ = 17
2	33.3			$J_{2a,3a}$ = 8, $J_{2a,3b}$ = 5
		2b	2.6	$J_{2b,3a}$ = 5, $J_{2b,3b}$ = 8
		3a	2.0	$J_{3a,3b}$ = 14
3	28.8			$J_{3a,4}$ = 4
		3b	2.2	$J_{3b,4}$ = 6
4	65.7		4.4	$J_{4,7}$ = 1
5	171.5			
6	113.9			
7	57.1		4.6	$J_{7,8a}$ = $J_{7,8b}$ = 2
		8a	1.6	$J_{8a,8b}$ = 15
8	31.7			$J_{8a,9}$ = 12
		8b	2.0	$J_{8b,9}$ = 2
9	77.7		4.1	$J_{9,10}$ = 7
10	73.2		3.7	$J_{10,11}$ = 7
11	32.4		1.3, 1.6	
12	25.1		1.4, 1.6	
13	29.2		1.2	
14	32.2		1.2	
15	22.6		1.2	
16	14.0		0.9	$J_{15,16}$ = 7

[a]Assignments are based on ^{1}H-^{13}C and ^{1}H-^{1}H correlation and ^{1}H decoupling experiments.

[b]Obtained at 75 MHz, $CDCl_3$. Multiplicities were determined from DEPT/90° and DEPT/135° experiments.

[c]^{13}C-^{1}H shift correlation by long-range coupling for J = 5 and J = 10 Hz gave the following results: C-1 (H-2a, H-2b); C-5 (H-4, H-7); C-6 (H-7,H-8b); C-7 (H-8a,H-8b); C-9 (H-7); C-11 (H-9); C-14 (H-16); C-15 (H-16).

to another methylene group (δ 2.3 and 2.6) that had J_{gem} = 18 Hz expected for a methylene α to a carbonyl group. Because determination of the relative configuration of **2** necessitated accurate values of coupling constants, computer simulation (PANIC) of the spin patterns containing H-7–H-4–(H-3)$_2$–(H-2)$_2$, on the one hand, and H-4–H-7–(H-8)$_2$–H-9–H-10–(H-11)$_2$ in the triacetate **4** was undertaken. The parameters thus obtained were in excellent agreement with those obtained from proton decoupling experiments on **4**. Furthermore, the similarity of the coupling constants established for **4** with those obtained from decoupling measurements on **2** indicates minimal conformational difference between the two (the only major change being observed for $J_{9,10}$, which changed from 7 Hz in **2** to 4 Hz in **4**). The evidence presented so far allows the allocation of $C_{11}H_{15}O_5$, the remaining C_5H_{11} being assigned to a pentyl chain as required from the remaining resonance signals (4 triplets and one quartet) in the ^{13}C-nmr spectrum of **2**. The results of ^{1}H-^{13}C correlation measurements, including a number of long-range ^{1}H-^{13}C correlations, are incorporated in Table 4. The major metabolite from the isolate of *T. koningii* is, thus, shown to have structure **2**.

With the aim of determining the relative configuration of **2** by X-ray diffraction methods, several attempts were made to grow suitable crystals of **2**, its triacetate derivative **4**, and its monoacetate derivative **5**, but all were unsuccessful. The product obtained by treating **2** with *p*-bromosulfonylhydrazine was a glass. Because the amount of **2** available was limited, the determination of the relative configuration was attempted by spectroscopic methods.

The conformation of the dihydropyran ring is disclosed by the coupling constants between H-7, H-8a, H-8b, and H-9 in **2** and **4** (Table 4, 5), which essentially locates

TABLE 5. ^{1}H- and ^{13}C-nmr Data of the Triacetate **4**.[a]

Position	δ ^{13}C[b]	Position	δ ^{1}H[c]	$J_{H,H}$ (Hz)[c]
1	194.8			
		2a	2.38	$J_{2a,2b}$ = 16.9
2	31.6			$J_{2a,3a}$ = 6.3, $J_{2a,3b}$ = 4.9
		2b	2.64	$J_{2b,3a}$ = 9.4, $J_{2b,3b}$ = 5.7
		3a	1.6	$J_{3a,3b}$ = 14
3	26.4			$J_{3a,4}$ = 4.7
		3b	2.2	$J_{3b,4}$ = 4.7
4	66.5		5.65	$J_{4,7}$ = 1
5	170.4			
6	112.1			
7	59.6		5.81	$J_{7,8a}$ = 3.5, $J_{7,8b}$ = 2.1
		8a	1.75	$J_{8a,8b}$ = 15
8	29.1[d]			$J_{8a,9}$ = 3.5
		8b	1.99	$J_{8b,9}$ = 2.2
9	74.2		4.15	$J_{9,10}$ = 3.9
10	72.9		5.08	$J_{10,11}$ = 6.7
11	29.9[d]		1.3, 1.6	
12	25.1		1.25	
13	29.2		1.25	
14	32.8		1.25	
15	22.5		1.25	
16	14.0		0.9	$J_{15,16}$ = 6.5

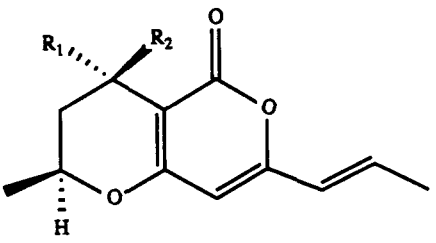
[a]Other signals: acetate carbons, δ ^{13}C 169.8, 168.4 (2), 21.2, 20.9, 20.8; acetate methyl protons, δ ^{1}H 2.14, 2.08, 2.05.

[b]75 MHz; $CDCl_3$.

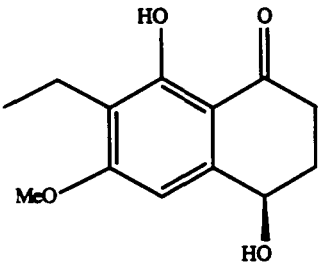
[c]Assignments are based on ^{1}H decoupling experiments. Values for $J_{H,H}$ were obtained from simulated (PANIC) spectra and were similar to those obtained from decoupling experiments.

[d]Values may be interchanged.

H-7 and H-9 in a *trans* relationship. NOe difference spectroscopy measurements on **2** and **4** gave the following results: Irradiation of H-7 gave significant nOe interactions with H-8a (15%) and H-8b (7%) confirming the conclusion that it subtends almost similar dihedral angles to the two protons. H-9 interacts with H-10 (12%) and with H-8b (5%), to which it has a small coupling ($J = 2$ Hz) and to the two hydrogens at C-11 (15%). In turn H-10, apart from an interaction with H-9, shows an nOe with H-8b (5%) and the two hydrogens at C-11 (15%). These results support the relative configuration shown in **B**. The coupling constants observed for the H-7 to H-9 spin pattern are similar to those obtained for the equivalent set of protons in 3-*epi*-deoxyradicinol [**6**] but differ from those of deoxyradicinol [**7**] (8,9).



	R_1	R_2
6	OH	H
7	H	OH



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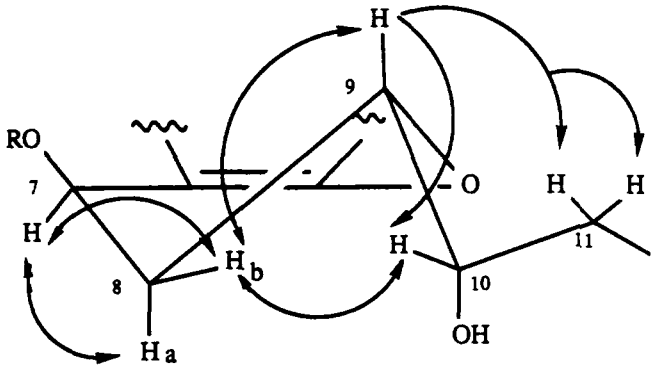

B

The conformation of the cyclohexenone ring also can be deduced from the coupling constants obtained for the H-2 to H-4 spin pattern and by comparison with a model system. That H-4 subtends similar angles to the two hydrogens at C-3 is revealed by the values of $J_{3a,4}$ and $J_{3b,4}$: 4 Hz, 6 Hz in **2**, respectively, and 4.7 Hz, 4.7 Hz in **4**. Furthermore, the absence of a $J > 10$ Hz between the C-2 and C-3 hydrogens argues against any two vicinal hydrogens being in a *trans*-diaxial relationship. This indicates that the cycohexenone ring in **2** adopts the half-boat rather than the half-chair conformation and in this way resembles more *O*-methylasparvenone [**8**] (10) than **3** in which $J_{4,5b} = 12.5$ Hz (7).

In the case of **2** two half-boat conformations are possible and a distinction between them, which would allow the relative configuration at C-4 to be assigned, is not readily made. In the event stepwise addition of $Eu(fod)_3$, a lanthanide shift reagent (LSR), to an nmr solution of **4** in $CDCl_3$ produced some significant results. The signal for H-7

moved rapidly to lower field with $\Delta = 19.5$ ($\Delta = \Delta\delta \cdot [\mathbf{4}]/[\text{LSR}]$) (11) followed by the signals for H-2a and H-2b, $\Delta = 6.2$ and 5.9, indicating that complexation with the carbonyl oxygen is preferred. The lanthanide-induced shifts (LIS, Δ) of other protons were H-9 (5.1), H-8b (5.0), acetate 2 (5.1), H-4 (3.4), H-8a (3.4), H-10 (3.2), H-3a and H-3b (2.2), acetate 1 (1.5), and acetate 3 (1.5). Acetate 1, which appears in the undoped spectrum at δ 2.14, is that at C-4 since the 4-monoacetate [**5**] shows a signal at a similar chemical shift. Acetate 3 (δ 2.05) has a comparable LIS value to that of the C-4 acetate and is assigned to C-10 since H-4 and H-10 also have similar LIS values, 3.4 and 3.2, respectively. Acetate 2 (δ 2.08), is therefore associated with H-7, and the pronounced LIS associated with these two sets of protons suggests that a bidentate complex between the C-1 carbonyl and the C-7 acetate is involved (12,13). This possibility requires that the two groups approach coplanarity. Because the conformation of the cyclohexenone ring appears to be essentially unchanged at low ratio of LSR to substrate ($\leqq$0.4), as indicated by the coupling constants of the $(\text{H-2})_2$–$(\text{H-3})_2$–H-4 spin system, the Dreiding model indicates that the acetates at C-7 and at C-4 and the C-1 carbonyl are all in a *syn* relationship. Thus, the major metabolite of *T. koningii* is tentatively assigned the relative stereochemistry shown in **2**.

With reference to the major lipophilic metabolites **1** and **2** produced by the two isolates of *T. koningii* examined by us, two points are worth noting. First, each isolate produces only one of the two metabolites with no obvious amount of the other. Second, although **1** is clearly a pentaketide, which appears to co-occur with traces of the hexaketide-derived analogue (4), and **2** is an octaketide, the origin of **2** from a precursor of **1** can be delineated.

EXPERIMENTAL

GENERAL EXPERIMENTAL METHODS.—^{1}H-nmr and 75 MHz ^{13}C-nmr spectra were recorded at 300 MHz on a Brucker AM-300 Spectrometer. Uv spectra were obtained using a Hewlett-Packard 8450 A UV/VIS Spectrophotometer. Mass spectra were measured with a Hewlett-Packard 5986 GC/MS System (35 eV). Hrms were recorded on a Varian MAT-31 Spectrometer. [α]D were measured using a Perkin-Elmer 141 Polarimeter with a 1 dm cell. For tlc Kieselgel 60 F_{254} aluminum sheets (Merck) were used.

ISOLATION OF *T. KONINGII* FROM SOIL.—The culture of *T. koningii* producing compound **2** was isolated from soil as described by Simon and Sivasithamparam (4). It was identified by the Commonwealth Mycological Institute and given the IMI No. 308477.

PREPARATION OF BROTH CULTURE OF *T. KONINGII*.—Sterile ⅕ strength Potato Dextrose Broth (PDB) (30 ml) was inoculated with a plug (7 mm diameter) of *T. koningii* taken from the growing edge of a culture on ⅕ strength Potato Dextrose Agar (PDA). The still broth culture was incubated at 20°, and when the fungal pellicle had covered the surface of the broth, the culture was shaken to break up the hyphae and 3 ml transferred to 400 ml of ⅕ PDB. The broth was agitated on an orbital shaker at 15° and 100 pm for 7 days and then filtered (Whatman 4) to remove most of the hyphae.

INHIBITION OF *GGT* BY BROTH CULTURE.—Broth culture (2 ml), sterilized by passage through 0.22 μm filter membrane, was transferred to a Petri dish. To the Petri dish was added ⅕ PDA (2% agar) (25 ml), and the contents were mixed by gentle agitation. The controls were (a) 22 ml unamended ⅕ PDA and (b) 2 ml filter-sterilized ⅕ PDA + 20 ml unamended ⅕ PDA. A plug (7 mm diameter) cut from the growing edge of a colony of *Ggt* (isolate WUF 271) on ⅕ PDA was placed in the center of the dish. There were five replicates of each treatment. The growth of the pathogen was measured as the area of the colony after 2,3, and 4 days incubation at 20°.

INHIBITION OF SOILBORNE PLANT PATHOGENS IN AGAR CULTURE.—Single thickness dialysis membrane (Type 45311, Union Carbide Corporation) was cut into pieces approximately 80 mm × 80 mm, boiled in 0.01 mM ethylenediaminetetraacetic acid, rinsed in de-ionized H_2O, and autoclaved in de-ionized H_2O at 121° for 20 min. A piece of membrane was placed on the surface of ⅕ PDA in a Petri dish (86 mm diameter). The center of the dish was inoculated with a plug (5 mm diameter) taken from the growing edge of a colony of *T. koningii* on ⅕ PDA. After 4 days incubation at 15°, the dialysis membrane was removed and the center of the dish inoculated with a plug (7 mm diameter) cut from the growing edge

of a culture of one of the following soilborne plant pathogens: *Ggt*, *Rhizoctonia solani* Kühn [Anastomosis group (Ag)-8], *Phytophthora cinnamomi* Rands (IMI No. 165644), *Pythium middletonii* Sparrow (IMI No. 309481), *Fusarium oxysporum* Schlecht. emend Snyder & Hansen, and *Bipolaris sorokiniana* (Sacc.) Shoem. There were three replicates of each treatment. The growth of the pathogens was measured as colony area after 1, 2, and 3 days incubation at 20°.

ISOLATION OF THE MAJOR METABOLITE FROM THE BROTH CULTURE OF *T. KONINGII*.—The culture broth (400 ml) was extracted repeatedly with EtOAc, and the combined organic layer was dried over Na_2SO_4 and evaporated under reduced pressure. The extract (100 mg) on tlc analysis (Si gel; EtOAc) appeared to contain one major (R_f 0.13) and several minor components which were uv active. Chromatography on neutral Al_2O_3 (activity I) and elution with EtOAc gave the major coupound **2** (2 mg) which crystallized from $CHCl_3$/pentane as solvated needles which collapsed to a powdery solid on recovery. The triol **2** had mp 122–123°, [δ]D + 166.9° (c = 0.3; $CHCl_3$). Found $[M]^+$ 298.1759; $C_{16}H_{26}O_5$ requires $[M]^+$ 298.1780. Ir ν max (CCl_4) (cm^{-1}) 3600, 3380, 1655, 1620 cm^{-1}; ν max ($CHCl_3$) 3600, 3400, 1650, 1620 cm^{-1}; λ max (EtOH) 260 nm (log ε 3.9); ^1H and ^{13}C nmr see Table 1; eims m/z $[M]^+$ 298 (3), 280 (5), 262 (4), 244 (8), 236 (6), 209 (13), 191 (7), 181 (12), 170 (18), 165 (39), 154.9847 [$C_7H_7O_4$ requires 154.9841] (100), 139 (78), 126 (36), 85 (37).

BIOASSAY OF ACTIVITY OF COMPOUND **2**.—A plug (7 mm diameter) was cut from the growing edge of a colony of *Ggt* on ⅕ PDA and placed in the center of a Petri dish containing ⅕ PDA. The sample of **2** obtained by cc was dissolved in 1ml EtOH, and 10 μl was dispensed directly on top of the inoculum plug. The lid of the dish was partially removed and the EtOH evaporated in a laminar flow cabinet. There were three replicates of each treatment. The dishes were incubated at 20° and the growth of the pathogen measured as area of the colony after 2, 3, 4, and 5 days incubation.

DERIVATIVES OF **2**.—*Monoacetate* **5**.—A solution of **2** (17 mg) in CH_2Cl_2 was treated with pyridine (1 drop) and Ac_2O (0.5 ml) and left for 4 h. The product recovered contained (tlc) one major and one minor component. Separation by preparative tlc (Si gel; EtOAc) provided the major compound, the monoacetate **5** (4 mg), as a gum (found 280.1668; $C_{16}H_{24}O_5$ $[M-60]^+$ requires 280.1674). ^1H nmr (300 MHz, $CDCl_3$) δ 5.6 (1H, dt, $J_{3a,4}=J_{3b,4}=4.6$ Hz, $J_{4,7}=1$ Hz, H-4), 4.7 (1H, ddd, $J_{4,7}=1$ Hz, $J_{7,8a}=4.1$ Hz, $J_{7,8b}=2.3$ Hz, H-7), 4.06 (1H, ddd, $J_{8a,9}=12$ Hz, $J_{8b,9}=2.4$ Hz, $J_{9,10}=4.1$ Hz, H-9), 3.6 (1H, dt, $J_{9,10}=4.1$ Hz, $J_{10,11}=7$ Hz, H-10), 2.6 (1H, ddd, $J_{2a,2b}=17.1$ Hz, $J_{2b,3a}=9$ Hz, $J_{2b,3b}=6$ Hz, H-2b), 2.4 (1H, ddd, $J_{2a,2b}=17.1$ Hz, $J_{2a,3a}=6$ Hz, $J_{2a,3b}=5.9$ Hz, H-2a), 2.18 (3H, s, acetate), 2.2 (1H, dddd, $J_{3a,3b}=14$ Hz, $J_{2b,3b}=6$ Hz, $J_{2b,3a}=9$ Hz, $J_{3b,4}=4.6$ Hz, H-3b), 1.97 (1H, ddd, $J_{7,8b}=2.3$ Hz, $J_{8a,8b}=14.5$ Hz, $J_{8b,9}=2.4$ Hz, H-8b), 1.85 (H, ddd, $J_{7,8a}=4.1$ Hz, $J_{8a,8b}=14.5$ Hz, $J_{8a,9}=12$ Hz, H-8a), 0.88 (3H, t, $J_{15,16}=6.5$ Hz, H-16$_3$); eims m/z $[M-60]^+$ 322 (1), 280 (6), 262 (10), 165.0547 [$C_9H_9O_3$ requires 165.0552] (100), 157 (19), 155 (21), 148 (30), 140 (11), 139 (29), 138 (21), 137 (25), 121 (11), 85 (16), 81 (10), 69 (19).

Triacetate **4**.—Treatment of **2** in pyridine with Ac_2O for 16 h afforded the triacetate **4** as a colorless oil. ^1H and ^{13}C nmr see Table 2; eims m/z $[M-43]^+$ 381 (20), 322 (56), 321 (52), 279 (27), 262 (45), 261 (31), 233 (49), 207 (38), 205 (13), 197 (16), 191 (18), 177 (11), 165 (100), 155 (33), 148 (66), 137 (31), 109 (24), 57 (44).

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