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METABOLITES OF *ASPERGILLUS TERREUS* ANTAGONISTIC TOWARDS THE TAKE-ALL FUNGUS

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ABSTRACT.—Terrein [**1**] and *E*-4-(1-propen-1-yl)-cyclopenta-1,2-diol [**2**] were isolated from standing cultures of *Aspergillus terreus*. Evidence for the structure of **2** is presented. Both metabolites inhibit the growth of wheat take-all fungus.

Most species of *Aspergillus* are encountered in soil as saprophytic organisms. They are efficient soil colonizers and are frequently isolated from soil debris (1,2). Species of *Aspergillus* are known pathogens of wheat, rye grass, and other plants (1,2). As part of a project aimed at the identification of fungi in roots of wheat and rye grass, five species of *Aspergillus* were isolated, with *Aspergillus terreus* Thom (Deuteromycotina) being isolated most frequently (2). Although *A. terreus* was shown to kill most wheat and rye grass seedlings at high inoculum levels (1%), with lower levels (0.5%) little or no effect was observed. Futhermore, at these low levels *A. terreus* reduced the root rot of wheat and rye grass caused by the take-all fungus [*Gaeumannomyces graminis* (Sacc.) von Arx & Oliver var *tritici* Walker (Ggt)] in sterilized and unsterilized soil. Because this isolate of *A. terreus* also inhibited the growth of Ggt in vitro (2) we decided to investigate the secondary metabolites produced by cultures of this fungus. We now report the isolation of terrein [**1**] and a new compound **2** from standing cultures of *A. terreus*. Both metabolites show antagonism towards Ggt.

Extraction of the liquid medium from standing cultures of *A. terreus* with EtOAc gave an extract that appeared from tlc to contain two major metabolites. Chromatography of the extract afforded, in order of decreasing polarity, the known metabolite terrein [**1**] and a new compound **2** whose structure was deduced as follows.

The compound had a molecular formula $C_8H_{14}O_2$ ($[M]^+$ 142) and showed absorption bands in its ir spectrum at 3560 and 3440 cm^{-1}, indicative of hydroxyl groups. The ^{13}C-nmr spectrum showed only six signals, suggesting that the compound had an element of symmetry, and included signals attributable to a 1-propenyl group (δ_C 17.66, q; 123.54, d; 135.51, d) oxymethine carbons (δ_C 73.06, d), methylene carbons (δ_C 38.59, t), and one methine carbon (δ_C 36.71, d). The ^1H-nmr spectrum and decoupling experiments revealed the presence of an *E*-1-propenyl system [δ_H 1.62 (d, $J=4.8$ Hz), 5.39 (dq, $J=4.8$, 15 Hz), 5.43 (dd, $J=6$, 15 Hz)]. The proton at δ 5.43 was coupled to an apparent sextet at δ 2.36 ($J=6$, 8, 9.5 Hz), which showed a coupling of 8 Hz to a two-proton signal at δ 2.1 (ddd, $J=5$, 8, 13.5 Hz) and a larger coupling (9.5 Hz) to another two-proton signal at δ 1.45 (ddd, $J=5$, 9.5, 13.5 Hz). Each of these sets of protons showed coupling to a signal at δ 4.0 (2H, br t, $J=5$ Hz) attributed to oxymethine protons. These results can be interpreted in terms of the gross structure shown in **2**. The syn relationship of the two hydroxyl groups was established directly by formation of the acetonide **3** and indirectly from the observation that **2** was optically inactive. The relative configuration shown in **2** was deduced from nOe difference experiments, which showed small (ca. 5%) but significant interactions between the 3β, 5β protons (at δ 1.45, shielded by the syn C-O bonds) and H-1′/H-2′ and between the 3α, 5α protons (δ 2.1) and the oxymethine protons (H-1 and H-2).

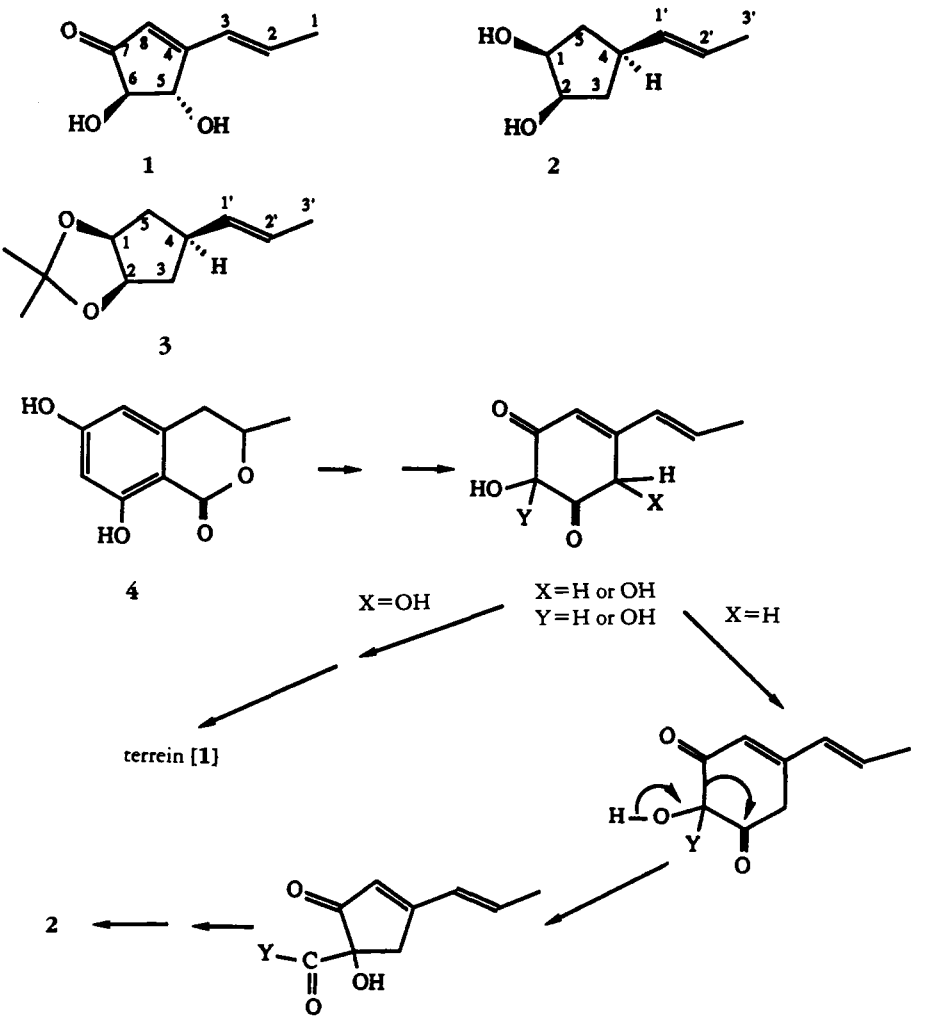
The origin of **2** is difficult to rationalize. It could be a catabolic product of terrein which accumulates in aged cultures. Given the present evidence (3) for the biosynthesis of terrein, **2** is unlikely to be a precursor of **1**. A third possibility is that **2** may arise from the proposed precursor of terrein, the lactone **4**, and reflect the partitioning of **4** between two pathways (Scheme 1) whose relative importance is a function of the age of the culture.

Isolates of *A. terreus* have been shown to be antagonistic towards the take-all fungus Ggt in vitro, to provide some protection to wheat plants from Ggt (1), and to produce metabolites that inhibit the growth of Ggt (4). We thus decided to test terrein and **2** for antagonism towards Ggt using a bioassay described previously (5). The results obtained (Table 1) indicate that both compounds inhibit the growth of Ggt over the shorter period of time but their effect diminishes over 5 days.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Methods, including preparation of broth culture and bioassays of compounds **1** and **2** with Ggt, are as described previously (5).

EXTRACTION AND ISOLATION.—Liquid medium (1.4 liters), obtained from a 3-month-old standing culture of an isolate of *A. terreus* Thom (deposited with the International



SCHEME 1. Postulated pathway for the biosynthesis of **2**.

TABLE 1. Inhibition of growth of *Gaeumannomyces graminis* var. *tritici* (Ggt) in Vitro by Compounds **1** and **2** from a Broth Culture of *Aspergillus terreus*.[a]

Compound[b]	Concentration (mg ml^{-1})	Growth Period of Ggt (days at 20°)	
		3	5
1	3.5	10.3 (0.5)	37.0 (1.2)
1	2.0	23.0 (0.8)[c]	38.7 (1.5)
2	2.0	21.0 (0.5)	34.7 (0.3)
Control[d]	—	29.7 (1.9)	43.0 (1.2)

[a]Measured as diameter of colony in mm on 1/10 strength Trypsic Soy Agar. Values are means of three replicates with SEM given in parentheses.

[b]Compound was dissolved in 1 ml of 10% EtOH/H_2O solution, and 40 µl of the solution was applied directly onto the inoculum plug of Ggt (9 mm).

[c]Mean of six replicates.

[d]40 µl of 10% EtOH/H_2O solution applied directly onto plug of Ggt (9 mm).

Mycological Institute, Kew, England, number 310164) (2), after filtration to remove the mycelial mat, was extracted with EtOAc. The organic layer was dried over Na_2SO_4 and evaporated in vacuo. The crude extract (0.85 g) contained two major components (R_f 0.3 and R_f 0.1) as determined by tlc [EtOAc-CH_2Cl_2 (1:4), Si gel]. These could be separated by radial plate chromatography (2 mm silica plate; CH_2Cl_2/EtOAc, gradient from 50% to 100% EtOAc). Filtration of each fraction through charcoal-Si gel gave compounds **1** and **2**. *E*-4-(1-propenyl-1-yl)-cyclopenta-1,2-diol [**2**] (89 mg) was isolated as a viscous oil, R_f 0.3, transparent to light from 589 to 365 nm. ^1H and ^{13}C nmr are reported in text; ir ($CHCl_3$) 3560, 3440, 960 cm^{-1}; eims *m/z* (% rel. int.) $[M]^+$ 142 (24), 124 (85), 109 (100), 106 (11), 97 (58), 96 (25), 95 (46), 83 (60), 69 (42). Terrein [**1**] (144 mg): mp 125–127° [lit. (6) mp 121–122°]; ir ($CHCl_3$) 3350, 1700, 1635 cm^{-1}; ^1H nmr (300 MHz, $CDCl_3$) δ_H 6.81 (dq, J = 15.8, 6.9 Hz, H-2), 6.40 (br d, J = 15.8 Hz, H-3), 6.02 (s, H-8), 4.89 (d, J = 2.3 Hz, H-5), 4.29 (d, J = 2.3 Hz, H-6), 1.97 (dd, J = 1.0, 6.9 Hz, H-1). For spectral data obtained for Me_2CO-d_6 and D_2O solutions see Hill *et al.* (3) and Dunn *et al.* (6), respectively. Eims *m/z* (% rel. int.) $[M]^+$ 154 (5), 139 (100), 121 (43), 111 (19), 109 (25), 97 (11), 95 (20), 79 (60). The mycelium was homogenized in MeOH, and the extract thus obtained was re-extracted with EtOAc. The fraction soluble in EtOAc was shown by tlc to contain further amounts of **1** and **2** (0.22 g) in a 1:1 ratio.

FORMATION OF ACETONIDE.—A solution of **2** (50 mg) in 2,2-dimethoxypropane (2 ml) was treated with camphor sulfonic acid (10 mg) and stirred for 2 h at room temperature. Excess K_2CO_3 was added, the solution was filtered, and the solvent was removed in vacuo. The residue in Et_2O was filtered through a plug of Si gel to give the acetonide **3** (30 mg), oil; *m/z* $[M-15]^+$ 167; ^1H nmr (300 MHz, $CDCl_3$) δ_H 5.56 (ddq, J = 15.1, 7.6, 1.4 Hz, H-1'), 5.40 (ddq, J = 15.1, 0.8, 7.1, Hz, H-2'), 4.62 (m, H-1, H-2), 2.54 (apparent sextet, individual peaks of the multiplet were broad, $W_{h/z}$ = 4 Hz, H-4), 2.0 (m, H_2-3, H_2-5), 1.64 (ddd, J = 7.1, 1.4, 0.8 Hz, H_3-3'), 1.30 and 1.26 (s, acetonide methyls); ^{13}C nmr (75.5 MHz, $CDCl_3$) δ_C 134.7 (d, C-2'), 123.7 (d, C-1'), 111.3 (s, acetal carbon), 81.0 (d, C-1, C-2), 42.0 (d, C-4), 39.0 (t, C-3, C-5), 27.0 (q) and 24.4 (q) (acetonide methyl carbons), 17.8 (q, C-3'); eims *m/z* (% rel. int.) $[M-15]^+$ 167 (100), 125 (15), 107 (61), 91 (19), 85 (19), 79 (72), 68 (14), 67 (18), 59 (12), 43 (20).

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