Koninginin G, a New Metabolite from *Trichoderma aureoviride*

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A new metabolite, koninginin G (**1**), was isolated from a strain of *Trichoderma aureoviride* and its structure established by the interpretation of spectroscopic data. The metabolite significantly inhibited the growth of etiolated wheat coleoptiles by 56% at 10^{-3} M.

During the course of examining fungi for biologically active natural products, we accessed a strain of *Trichoderma aureoviride*. Upon subsequent semisolid fermentation, compound **1** was isolated using an etiolated wheat coleoptile bioassay to detect activity in the crude extract and to direct the fractionation. This bioassay has been successfully used to detect, and isolate, koninginins A,[1] B,[2] C,[3] and E[4] from *Trichoderma koningii*. Furthermore, it has detected antimicrobials, immunosuppressants, plant growth regulators, and mycotoxins.[5,6] Although it may be argued, however, that certain compounds exhibit activity only at concentrations of 1 mM, the system is simply an indicator of activity, and it has been our experience that bioactive compounds are amplified when placed in their correct biological niche. For example, the natural product fungicide, 6-pentyl-α-pyrone, only inhibited coleoptiles (100%) at 10^{-3} M, but in very dilute solutions it was highly active against *Aspergillus flavus* in disk assays.[7] In contrast, Ghisalberti isolated koninginins D and F from a strain of *Trichoderma harzianum* and reported their activities against *Gaeumannomyces graminis* var *tritici*.[8,9]

LRCIMS of **1** showed a molecular ion peak at *m/z* 302, suggesting the molecular formula $C_{16}H_{30}O_5$ and two degrees of unsaturation. The IR spectrum (KBr) showed absorption bands at 3518, 3482, and 3382 cm^{-1}, indicating the presence of hydroxyl groups. Significantly, the ^1H and ^{13}C NMR spectra suggested a close similarity to the known koninginin A reported by Cutler and co-workers in 1989 from *T. koningii*.[1] In addition, the DEPT 135° spectrum indicated the presence of one methyl, nine methylenes, five methines, and one quaternary carbon. A D_2O exchange experiment showed the presence of four exchangeable hydroxyl signals (Table 1). Acetylation of **1** at room temperature afforded the tri-*O*-acetate derivative **2**, which indicated that three of these exchangeable signals were secondary alcohols. In the ^1H NMR spectrum of **1** a proton doublet of doublets resonating at δ 3.54 (Table 1), which correlated to the oxygenated methine carbon at δ 73.6, was assigned to H-4. The proton doublet of double doublets resonating at δ 3.76 correlated to the oxygenated methine carbon at δ 74.0 (Table 1) and was assigned to H-9. This proton showed $^1H-^1H$ COSY coupling to another oxygenated proton doublet of doublets, which absorbed at δ 3.66 and which correlated to the methine carbon at 69.5 ppm, and was assigned as H-10. The quaternary carbon that absorbed at δ 97.0 was assigned to C-5, based on the HMBC correlations with H-1 and H-9 (Figure 1). The upfield shift of the H-9 and H-10 signals from those of koninginin A (−0.56 and −0.64 ppm, respectively) suggested the opening of the C-5−C-10 ether bridge of koninginin A with the addition of one molecule of water. This was further confirmed by the upfield shift of carbons C-5, C-9, and C-10 (−12.2, −5.0, and −9.8 ppm, respectively) and the downfield shift of C-4 (+3.8 ppm), as compared to those of koninginin A.[1] Further, evidence for the structure of **1** was provided by the LR−COSY correlations between the C-5

Table 1. ^{13}C and ^1H NMR Spectral Data of **1**[a]

position	^{13}C	^1H
1	74.6, d	3.39, dd (7.5, 6.9)
2	32.1, t	1.47, m
		1.33, m
3	24.8, t	1.81, m
		1.69, m
4	73.6, d	3.54 dd (10.7, 5.3)
5	97.0, s	
6	43.4, d	1.38, m
7	24.9, t	1.89, m
		1.72, m
8	27.6, t	2.08, dddd (17.8, 13.1, 9.3, 3.6)
		1.43, m
9	74.0, d	3.76, ddd (12.6, 9.5, 1.8)
10	69.5, d	3.66, dd (10.0, 1.8)
11	29.4, t	1.85, m
		1.42, m
12	20.8, t	1.52, 2H, m
13	31.5, t	1.36, m
		1.25, m
14	31.8, t	1.39, m
		1.26, m
15	22.6, t	1.50, 2H, m
16	14.1, q	0.87, t (6)
OH-1		4.38
OH-4		5.16, s
OH-5		5.17, s
OH-10		3.88, d (10.1)

[a] In $CDCl_3$, carbon multiplicities were determined by DEPT 135 experiments. s = quaternary, d = methine, t = methylene, q = methyl carbons; coupling constants (*J*) are in Hz.

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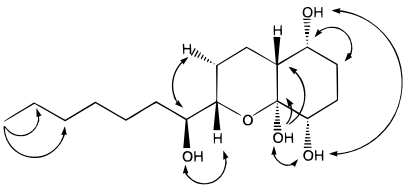
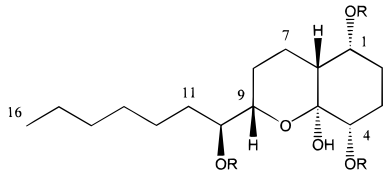


Figure 1. Significant HMBC, COSY (single arrow), and NOESY (double arrow) correlations of **1**.

hydroxyl singlet with H-6, proton H-9 with H_2-7 and H_2-11, and also the LR−COSY correlation between H-10 and H_2-8.

The relative stereochemistry of **1** was established based on the NOESY spectrum as well as comparison of the proton coupling constant values with koninginin A.[1] The proton H-10 was assigned with an α-orientation based on its *J* values (10, 1.8 Hz). In koninginin A, the H-10 proton absorbed at δ 4.24 as a narrow doublet (1.8 Hz) and was assigned as α.[1] H-10 also showed a strong NOESY correlation in **1** with the pseudoaxial α-oriented H-8a, which absorbed at δ 2.08 (Table 1). The C-10 β-OH doublet absorbed at δ 3.88 and showed a strong NOESY correlation with H-9, suggesting its β-orientation. Furthermore, *J* values of the H-4 proton in **1** (10.7, 5.3 Hz) were similar to that of H-4 in koninginin A (11.6, 5.4 Hz), suggesting that koninginin A and compound **1** have the same orientation at H-4. Also, H-10 showed strong NOESY correlations to the C-4 and C-5 hydroxy singlets, which suggested the same α-orientation as koninginin A. The NOESY correlation between H-1 and H-9 indicated that these protons are in the β-orientation in compound **1**. Because compound **1** was isolated for the first time from a natural source and is structurally similar to the koninginin class, it was given the name koninginin G.

Koninginin G significantly (p <0.01) inhibited the growth of etiolated wheat coleoptiles by 56% relative to controls, at a 10^{-3} M concentration.



Koninginin G **1** R = H

Koninginin G triacetate **2** R = Ac

Experimental Section

General Experimental Procedures. Melting points were determined on a Thomas−Hoover capillary melting point apparatus and are uncorrected. Optical rotations were measured with a JASCO-370 digital polarimeter. UV and IR spectra were acquired on Perkin−Elmer Lambda 4B UV/vis and Perkin−Elmer 16PC spectrometers, respectively. GC/MS were recorded on an HP 5970A MSD spectrometer interfaced to an HP 5890 GC using a DB-1 column, 15 m × 0.25 mm, 0.25 μm film thickness, column temperature 170 °C (1 min) to 270 °C programmed 30 °C/min, injector temperature 250 °C, detector temperature 280 °C, with helium as the carrier gas. LRCIMS were obtained on a Micromass Autospec double-focusing sector instrument, using *m*-nitrobenzyl alcohol as a matrix. The ^{1}H and ^{13}C NMR data were recorded with TMS as the internal standard, using a Bruker DRX-400 instrument operating at 400 and 100 MHZ. Si gel 60-F_{254} Merck was used for TLC.

Fungal Material. A strain of *Trichoderma aureoviride* Rifai was collected at Shannon, North Island, New Zealand, from necrotic stem tissue of *Salix matsudana* × *alba*,[10] and identification of the isolate was confirmed by the American Type Culture Collection.[11,12] The fungus was plated out on potato−dextrose agar, and a pure culture was obtained and stored at 5 °C. The fungus was inoculated in 50 mL of potato−dextrose broth and kept for 1 week in a stationary phase at 27 °C, then the mycelium- and spore-laden broth (1 mL) was seeded onto a medium in each flask consisting of 100 g shredded wheat, 200 mL Oxoid mycological broth (pH 4.8), 2% yeast extract, and 20% sucrose[13] in 2.8-L Fernbach flasks (54 flasks) followed by incubation for 14 days at 24 °C.

Extraction and Isolation. After incubation, 300 mL of Me_2CO were added to each flask, and the mycelia and the substrate were homogenized. The suspension was filtered, and the filtrate was concentrated under vacuum. The residue (120 g) was mixed with H_2O (300 mL), then extracted with EtOAc (600 mL × 2). The combined EtOAc extracts were dried over anhydrous Na_2SO_4, reduced in volume, and chromatographed over a Si gel column (365 g silica gel 60, 10 × 13 cm). The column was eluted with C_6H_6 (1 L), *tert*-butyl methyl ether (1 L), EtOAc (1 L), Me_2CO (1 L), and CH_3CN (1 L). Each fraction was bioassayed in the etiolated wheat coleoptile bioassay.[5,6] A white precipitate was separated from the Me_2CO fraction upon cooling and was crystallized from Me_2CO to yield 976 mg of **1**.

Koninginin G (1): mp 134−135 °C; $[\alpha]^{21}_D$ + 24° (*c*, 0.005, EtOH); UV λ_{max} (CH_3OH) (log ϵ) 212 (3.93), 260 (3.88) nm; IR (KBr) 3518, 3482, 3382, 2945, 2918, 2857, 1433, 1081, 953 cm^{-1}; ^{1}H and ^{13}C NMR see Table 1; GC−MS t_R 3.93 min, *m*/*z* 284 ($M^+ - H_2O$, 11), 266 ($M^+ - 2H_2O$, 6), 256(1), 248 ($M^+ - 3H_2O$, 9), 199 (10), 181 (M^+ − side chain, 100); HRFABMS *m*/*z* 302.2069 (M^+, calcd for $C_{16}H_{30}O_5$, 302.2093), 285.2067 ([M + H − H_2O]$^+$ calcd for $C_{16}H_{29}O_4$, 285.2064), 267.1949 ([M + H − $2H_2O$]$^+$ calcd for $C_{16}H_{27}O_3$, 267.1949), and 249.1880 ([M + H − $3H_2O$]$^+$ calcd for $C_{16}H_{25}O_2$, 249.1855); R_f 0.40 (toluene−EtOAc−HCOOH, 5:4:1, pink spot with *p*-anisaldehyde).

Koninginin G Triacetate (2). An equal mixture of Ac_2O and dry pyridine (2 mL) were added to 15 mg of **1** and kept at room temperature for 24 h. Usual workup and subsequent purification using preparative TLC (Si gel plate; hexane−Me_2CO, 3:2, band with R_f 0.35) furnished 10 mg of **2**: mp 121−122°, $[\alpha]^{21}_D$ +12.5° (*c*, 0.005, EtOH); ^{1}H NMR ($CDCl_3$) δ 5.15 (1H, dd, J = 9, 7.5 Hz, H-1), 5.04 (1H, dd, J = 11.3, 6.4 Hz, H-4), 4.92 (1H, d, J = 9.1 Hz, H-10), 2.15 (3H, s, OAc-1), 2.09 (3H, s, OAc-4), 2.08 (3H, s, OAc-10); ^{13}C NMR δ 171.2 s (CO), 170.7 s (CO), 170.1 s (CO), 76.3 d (C-1), 73.9 d (C-4), 73.4 d (C-9), 71.6 d (C-10), 53.9 d (C-6), 31.5 t (C-2), 30.7 t (C-14), 30.3 t (C-13), 28.9 t (C-11), 28.2 t (C-8), 27.1 t (C-7), 25.2 t (C-3), 22.4 t (C-15), 20.9 t (C-12), 20.9 q (OAc-1), 20.7 q (OAc-4), 20.4 q (OAc-10), 13.9 q (C-16); GC−MS t_R 6.28, *m*/*z* 428 (M^+, 0.4), 368 ($M^+ - CH_3COOH$, 17), 350 ($M^+ - CH_3COOH$, $- H_2O$, 6), 326 (5), 308 ($M^+ - 2CH_3COOH$, 39), 290 ($M^+ - 2CH_3COOH - H_2O$, 11), 266 (21), 253 (38), 248 ($M^+ - 3CH_3$-COOH, 86), 230 (31), 133 (100).

Bioassay. The biological effects were determined using etiolated wheat coleoptiles (*Triticum aestivum* L. cv. Wakeland).[14,15] All data were statistically analyzed.[16]

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