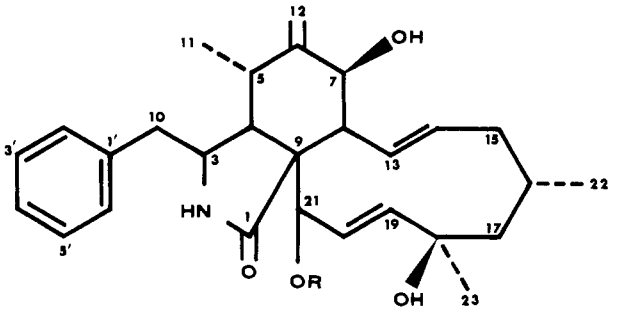
Isolation and Biological Properties of Deacetylcytochalasin H from *Phomopsis* sp.

The [11]cytochalasin deacetylcytochalasin H (7,18,21-trihydroxy-16,18-dimethyl-10-phenyl-[11]cytochalasa-6(12),*trans*-13,*trans*-19-trien-1-one) was isolated and identified from culture extracts of *Phomopsis* sp. by use of spectroanalytical techniques. Biological data presented an LD_{50} in chickens of a single oral dose at 37.5 mg/kg and plant growth inhibitory activity down to 10^{-5} M in wheat coleoptiles.

In previous communications, we reported the isolation, chemical characterization, and biological properties of a new [11]cytochalasin metabolite from culture extracts of *Phomopsis* sp. (Wells et al., 1976; Beno et al., 1977). The new metabolite [21-acetoxy,7,18-dihydroxy-16,18-dimethyl-10-phenyl-[11]cytochalasa-6(12),*trans*-13,*trans*-19-trien-1-one] was given the trivial name cytochalasin H (I). Further examination of cultural extracts of the same



I cytochalasin H, R = $CH_3C(=O)$-

II deacetylcytochalasin H, R = H

fungus produced a companion metabolite that was also an [11]cytochalasin (II). We wish to report the chemical structure of II derived via spectroscopic methods and some of its biological properties in plant and animal systems.

EXPERIMENTAL SECTION

Procedures for production and extraction of II were identical with those previously reported for cytochalasin H (Wells et al., 1976). Cytochalasin H and II were eluted together from silica gel 60 columns with ethyl ether. A second silica gel 60 column eluted with a linear gradient from *n*-hexane to ethyl ether effected separation of cytochalasin H from the more polar companion metabolite. The fractions containing II were combined, evaporated to dryness, and crystallized from ethyl ether solution at 5 °C.

Physical and Chemical Analyses. Purification of I and II was monitored by thin-layer chromatography (TLC) using 5 × 10 cm glass plates precoated with silica gel 60 F-254 (EM Laboratories, Inc.). The TLC developing solvent was toluene–ethyl acetate–formic acid, 5:4:1 (v/v/v). The cytochalasins were visualized on TLC plates by spraying with 50% ethanolic H_2SO_4 and heating at 100 °C for 3 min.

Melting points (mp) were determined on a Kofler micromelting point apparatus and were uncorrected. Ultraviolet spectra (UV) of I and II were recorded with a Beckman Model DB-G recording spectrophotometer in methanol solution at a concentration of 1.0×10^{-5} M.

Infrared spectra (IR) were recorded with a Perkin-Elmer Model 257 spectrophotometer equipped with a 4× beam condenser. Samples for analyses were coated onto KBr windows. Low-resolution (LRP) and high-resolution (HRP) mass spectra were obtained on a BG Micromass 70/70 spectrometer. Samples were introduced into the spectrometer via the direct-probe technique. NMR spectra were acquired in the FT mode on a Varian Associates XL-100-12 NMR spectrometer equipped with the 620L disk data system. Samples (20 mg/0.5 mL) were prepared in a $CDCl_3$–Me_2SO (75:25) solution with a small amount of Me_4Si added as an internal reference. Single-frequency, off-resonance proton-decoupled ^{13}C NMR spectra were obtained to aid in the assignment of the chemical shifts.

Animal and Plant Bioassays. The LD_{50} of II was determined in 1-day-old chickens dosed orally via crop intubation. The formulations of purified toxin for LD_{50} determination were prepared as previously described with 10 replications each at levels of 12.5, 25, 50, and 100 mg/kg (Kirksey and Cole, 1974). Weil's tables were used to determine the LD_{50} for II (Weil, 1952).

Four-day-old etiolated wheat seedlings (*Triticum aestivum* L. cv. Wakeland) that had been grown on moist sand in trays in the dark at 22 ± 1 °C were used to detect plant growth inhibition (Hancock et al., 1964). After their roots and caryopses were removed, seedlings were placed tip first into a Van der Weij guillotine. The apical 2 mm were discarded, and the next 4 mm were cut off and retained for bioassay. Metabolite II was prepared for bioassay by dissolving 4.51 mg in 50 μL of acetone (Cutler, 1968) and bringing the volume to 10 mL with phosphate–citrate buffer at pH 5.6 that contained 2% sucrose (Nitsch and Nitsch, 1956) to make a 10^{-3} solution. Serial dilutions were made from this at 10^{-4}, 10^{-5}, 10^{-6}, and 10^{-7} M. All these concentrations were bioassayed.

Ten 4-mm coleoptile sections were added to each test tube which contained 2 mL of buffer solution plus II. Each bioassay step was conducted under a green safelight (Nitsch and Nitsch, 1956). Finally, tubes were rotated at 0.25 rpm in a roller tube apparatus for 24 h at 22 °C in the dark. Coleoptiles were measured by projecting their images (×3) from a photographic enlarger (Cutler and Vlitos, 1962), and all data were analyzed statistically (Kurtz et al., 1965).

RESULTS AND DISCUSSION

Purified II formed large crystals from ethyl ether, mp 274–276 °C (partial decomposition), $[\alpha]^{27}_D = +47.8$ (c = 0.43 in methanol). It was visualized on TLC as a single rose-colored spot at R_f 0.44 compared to 0.53 for cytochalasin H. The UV spectrum of II was similar to that of I. Both showed strong end absorption and a series of weak bands in the region of 240–280 nm due to the $\pi \rightarrow \pi^*$ transition of the monosubstituted aromatic ring. Conspicuous in the IR spectrum of II was the absence of ester carbonyl absorption (1735 cm^{-1}), which was present in the IR spectrum of I.

The HRP spectrum of II gave a molecular ion peak at 451.2676 (calcd for $C_{28}H_{37}O_4N$ = 451.2722). In addition to the molecular ion peak, the LRP mass spectrum of II showed major peaks at m/e 415, 361, 342, 324, 270, 120, and 91 (base peak). The ^{13}C NMR spectrum of II (Table I), exhibited 26 peaks. Peak intensity considerations suggested that two of the peaks in the aromatic carbon region were due to two carbons each, typical of mono-

Table I

cytochalasin H ^{13}C NMR assignments[a]		deacetylcytochalasin H ^{13}C NMR assignments[a] (^{1}H NMR)[b]	
carbon	ppm	carbon	ppm
1	174.4 s	1	175.84 s
2		2	(6.64)
3	57.8 d	3	53.14 d (3.20)
4	50.3 d	4	49.23 d (2.97)
5	32.9 d	5	32.69 d (2.70)
6	148.2 s	6	149.72 s
7	69.8 d	7	70.05 d (3.94)
8	47.3 d	8	45.54 d (3.20)
9	51.9 s	9	53.24 s
10	42.9 t	10	42.91 t (2.70, 2.83)
11	14.0 q	11	13.72 q (0.87)
12	113.8 t	12	112.19 t (4.97, 5.21)
13	127.2 d	13	128.02 d (5.76)
14	138.3 d	14	137.24 d (5.31)
15	45.6 t	15	45.00 t (1.80)
16	31.2 d	16	32.69 d (1.75)
17	57.8 t	17	53.77 t (1.75)
18	74.2 s	18	73.42 s
19	127.0 d	19	136.37 d (5.83)
20	138.5 d	20	130.65 d (5.90)
21	77.5 d	21	75.71 d (4.52)
22	26.5 q	22	26.39 q (1.01)
23	28.5 q	23	28.19 q (1.28)
1′	137.4 s	1′	137.53 d
2′,6′	129.1 d	2′,6′	129.24 d (7.23)
3′,5′	128.9 d	3′,5′	128.24 d (7.23)
4′	125.9 d	4′	126.38 d (7.23)

[a] In ppm downfield from Me_4Si. Single-frequency, off-resonance proton decoupling results are given as s for singlet, d for doublet, etc. [b] Proton chemical shifts given in parentheses.

substituted benzene. The ^{1}H NMR spectrum of II (Table I) confirms the presence of a monosubstituted phenyl ring and suggests the presence of three methyl groups, a terminal double bond, and two trans double bonds. Comparison of all the analytical data of II with those of I confirm that II is deacetylcytochalasin H (7,18,21-trihydroxy-16,18-dimethyl-10-phenyl-[11]cytochalasa-6-(12),*trans*-13,*trans*-19-trien-1-one) (Table I).

Biological Activity. The LD_{50} of a single oral dose of II in day-old chickens was 37.5 mg/kg compared to 12.5 mg/kg for cytochalasin H.

Wheat coleoptiles were significantly inhibited ($P < 0.01$) and curved by solutions of deacetylcytochalasin H at 10^{-3}, 10^{-4}, 10^{-5}, and 10^{-6} M (Figure 1). Curvature of the coleoptiles was concentration dependent. Even at 10^{-3} M where there was 100% inhibition of growth, there was marked curvature. The relative inhibition values obtained for deacetylcytochalasin H at 10^{-3}, 10^{-4}, 10^{-5}, and 10^{-6} M were 100, 82, 40, and 16% compared to controls vs. 84, 80, 76, and 10% at 10^{-3}, 10^{-4}, 10^{-5}, and 10^{-6} M for cytochalasin H (Cutler et al., 1980). Further, deacetylcytochalasin H and cytochalasin H were more active than cytochalasins A, B, or E but were not as inhibitory to wheat coleoptile growth as chaetoglobosin K.

Patwardhan et al. (1974) repored the isolation of two new cytochalasin-type metabolites, designated Kodo-cytochalasin 1 and 2 from *Phomopsis paspali*. Kodo-cytochalasin 1 was subsequently shown by X-ray analysis to be identical with cytochalasin H (McMillan et al., 1977). Patwardhan et al. (1974) state that Kodo-cytochalasin 2 was deacetyl-Kodo-cytochalasin 1. Thus, Kodo-cytochalasin 2 should be identical with deacetylcytochalasin H. The analytical data presented by Patwardhan et al. (1974) are not sufficiently comprehensive for us to confirm that deacetylcytochalasin H from *Phomopsis* sp. is identical with Kodo-cytochalasin 2.

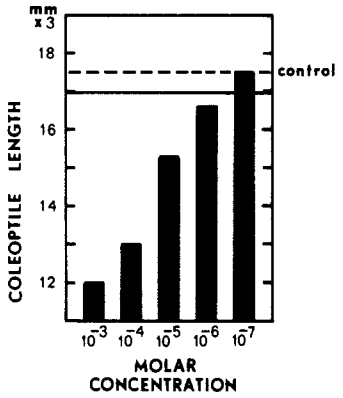


Figure 1. Growth-regulating activity of deacetylcytochalasin H in wheat coleoptile bioassays (*Triticum aestivum* L. cv. Wakeland). Control: dashed line. Inhibition is significant ($P < 0.01$) below the solid line.

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