# Proton and Carbon-13 Nuclear Magnetic Resonance Studies of the Conformation of Cytochalasin H Derivatives and Plant Growth Regulating Effects of Cytochalasins

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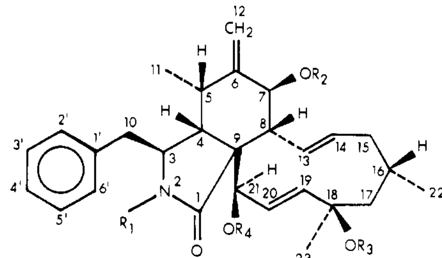
Analysis of the $^1$H and $^{13}$C NMR (nuclear magnetic resonance) spectra of acetate derivatives of cytochalasin H shows that acetylation of the nitrogen (N-2) produces a slight puckering of the five-membered ring. This in turn results in a shorter average distance between the phenyl ring and C-11 and an upfield shift of ~0.7 ppm for the protons on C-11. The role played by the hydroxyl groups in the expression of biological activity by cytochalasin H has been examined by comparing the plant growth regulating properties of the acetate derivatives of cytochalasin H. The results suggest that the hydroxyl group on C-18, and to a lesser extent the N–H group at N-2, is necessary for plant growth regulating properties.

Earlier, we reported the isolation, chemical characterization, and biological properties of two new [11]cytochalasin metabolites from culture extracts of *Phomopsis* sp. (Wells et al., 1976; Beno et al., 1977; Cole et al., 1981). The new metabolites, 7,18,21-trihydroxy-16,18-dimethyl-10-phenyl[11]cytochalasa-6(12),*trans*-13,*trans*-19-trien-1-one and 21-acetoxy-7,18-dihydroxy-16,18-dimethyl-10-phenyl[11]cytochalasa-6(12),*trans*-13,*trans*-19-trien-1-one, were given the trivial names deacetylcytochalasin H (1) and cytochalasin H (2), respectively, and are identical with kodocytochalasin-2 and kodocytochalasin-1, respectively (Patwardhan et al., 1974; McMillan et al., 1977). Because the cytochalasins elicit a wide range of plant growth regulating responses on intact plants (Wells et al., 1976) and in the wheat coleoptile bioassay (Wells et al., 1976; Cutler et al., 1980; Cole et al., 1981, 1982), it was decided to examine the roles played by the hydroxyl groups in the expression of biological activity (Cutler et al., 1978, 1979; Springer et al., 1981) by cytochalasin H (2). This was accomplished by preparing the 7-acetoxy (3), 2,7-diacetoxy (4) and 2,7,18-triacetoxy (5) derivatives of cytochalasin H and comparing the plant growth regulating properties of 1–5 with those of other cytochalasins.




|   | $R_1$ | $R_2$ | $R_3$ | $R_4$ |
|---|---|---|---|---|
| 1 | H | H | H | H |
| 2 | H | H | H | Ac |
| 3 | H | Ac | H | Ac |
| 4 | Ac | Ac | H | Ac |
| 5 | Ac | Ac | Ac | Ac |

During the course of this investigation, it became apparent that the introduction of additional acetate groups on cytochalasin H, particularly on N-2, was possibly introducing conformational differences among 1–5. Therefore, we have carried out a detailed NMR (nuclear magnetic resonance) investigation of 1–5 to define any conformational differences among 1–5. We report here the results of the NMR studies and the plant growth regulating properties of 1–5.

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## EXPERIMENTAL SECTION

**NMR Spectra.** Proton NMR spectra of chloroform-*d* solutions (~15 mg/0.5 mL) of 1–5 at 300 MHz were obtained on a NTC-300 spectrometer. The free induction decays (FID) were collected into 32K data points by using quadrature detection and a sweep width of 4000 Hz. The FID was zero-filled to 64K and resolution enhanced by using a double-exponential multiplication. Homonuclear decoupling experiments were conducted on 2 and 5 to aid in the assignment of the protons. In addition, two-dimensional $J$ correlated spectra of 2 and 5 were run using a 90–$\tau$–90 acquisition pulse sequence to establish the coupling relationships. A total of 512 spectra of 1K data points were collected for each of the two-dimensional experiments and the second dimension was zero-filled to give a 1K by 1K matrix.

Natural abundance, proton decoupled $^{13}$C NMR spectra were obtained on a Varian XL-100-15 spectrometer interfaced to a Nicolet 1280 data system. Single-frequency, off-resonance coupled (sford) spectra were run to aid in the assignment of the chemical shifts. Samples for $^{13}$C NMR spectra were made up to 100 mg/0.5 mL in chloroform in 5-mm sample tubes with a trace of $Me_4Si$ added as an internal reference. Typical parameters used to obtain the $^{13}$C NMR spectra were as follows: sweep width 5000 Hz; pulse angle 45°; data points 8K; delay between pulses 1 s; broad-band square-wave modulated proton decoupling; line broadening 1.0 Hz; quadrature phase detection.

Mass spectra were obtained with a VG Micromass 70/70 spectrometer in the EI mode and on a Varian Mat 112 spectrometer in the CI mode by using ammonia as the reagent gas. Samples were introduced via the direct probe. Infrared spectra were obtained with a Beckman IR 4210 spectrometer equipped with a 4X beam condenser by using thin films of the products on KBr windows. Uncorrected melting points were taken on a Kofler micromelting point block. For thin-layer chromatography (TLC), the compounds were developed on silcia gel 60 F-254 TLC plates, (E. M. Laboratories, Inc.) in ethyl acetate–benzene, 4:1 (v/v). The compounds were observed as dark spots at 254 nm.

**7-Acetoxycytochalalsin H (3).** Two hundred and fifty milligrams (0.507 mmol) of 2 were dissolved in 50.8 mg of acetic anhydride 0.507 mmol) and 1.0 mL of pyridine, in a centrifuge tube and were placed under nitrogen, overnight at room temperature. Cracked ice was added to the

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reaction mixture and allowed to melt, then ethyl acetate was added, and the mixture was stirred. The ethyl acetate fraction was removed, and the remaining mixture was further extracted twice with ethyl acetate. The ethyl acetate fractions were combined, dried over anhydrous sodium sulfate, and then taken to dryness under vacuum at 50 °C. The resulting syrup was placed on a silica gel chromatography column (25 × 15 cm, 70–230 mesh) packed in ethyl acetate–benzene, 4:1 (v/v). 7-Acetoxycytochalasin H (**3**) was associated with a blue-green fluorescent band when observed under long-wavelength UV and eluted from the column in the aforementioned solvent system to yield a white powder upon removal of the solvent (mp 174–176 °C; TLC $R_f$ 0.83–0.86; yield 51%). Low-resolution EI mass spectra yielded peaks at 535, 517, 475, 457, 444, 415, 397, 324, 306, 251, 120, and 91 (base). High-resolution EI data yielded 535.2938 (calculated for $C_{32}H_{41}O_6N$ 535.2933). CI mass spectra (reagent gas ammonia) gave prominent peaks at 553, 536, 518, 476 (base), and 416. Infrared data were 3500–3200 (flat-bottomed peak 3380–3200), 2960, 2915, 1725 (strong), 1685 (strong), 1430 (weak), 1365 (strong), 1222 (very strong), 1042, 1015 (strong), and 960 (strong), 902, 736, and 695 $cm^{-1}$.

**2,7-Diacetoxycytochalasin H (4) and 2,7,18-Triacetoxycytochalasin H (5).** In a 10-mL beaker were added 493 mg of **2** (1 mmol), 2.16 g (0.021 mol) of acetic anhydride, and 3 mL of dichloromethane. While being stirred, 183 mg (1.5 mmol) of 4-(dimethylamino)pyridine was slowly added. The reaction mixture was stirred at room temperature for 4 h and then reduced in volume, under vacuum at 50 °C, to dryness. The residue was added in ethyl acetate–benzene, 4:1 (v/v), solution to a silica gel (70–230 mesh) chromatography column (1 × 48 cm) packed in the ethyl acetate–benzene solvent mixture. Elution with this solvent yielded two blue-green fluorescent bands, visible under long-wave UV that were collected separately. The upper band (**5**), as observed by TLC ($R_f$ 0.93–0.96) eluted first and was followed by the second band (**4**) ($R_f$ 0.90–0.93). Evaporation of each eluate yielded a white powder (**4**, mp 107–111 °C and yield 191 mg, 33%; **5**, mp 103–105 °C and yield 242.4 mg, 42%). Chemical ionization mass spectra using ammonia as the reagent gas gave prominent peaks for **4** at 595 ($M^+ + NH_4$), 577, 560, 518 while **5** gave peaks at 637 ($M^+ + NH_4$), 619, 595, 577, and 560. The infrared spectrum of **4** gave bands at 3560–3300 (broad), 2960, 2920, 1720 (strong), 1696 (shoulder) 1442–1410, 1365 (very strong), 1270 (shoulder), 1222 (very strong), 1040, 1015 (strong), 960, 912, 738, and 700 $cm^{-1}$. For **5** peaks were observed at 2960–2920 (broad), 1720 (very strong), 1696 (shoulder), 1442–1412 (broad), 1365 (very strong), 1265 (shoulder), 1240 (shoulder), 1220 (very strong), 1110, 1040, 1015, 955, 912, 738, and 698 $cm^{-1}$.

**Bioassay.** Coleoptiles, each 4 mm long, were cut from 4-day-old etiolated wheat seedlings (*Triticum aestivum* L. cv. Wakeland) grown in the dark at 22 ± 1 °C. They were incubated in solutions of phosphate–citrate buffer at pH 5.6 that contained 2% sucrose (Nitsch and Nitsch, 1956) plus the compound to be tested. Each compound was dissolved in acetone (Cutler, 1968) and added to test tubes with buffer solution to produce concentrations of $10^{-3}$, $10^{-4}$, $10^{-5}$, and $10^{-6}$ M. Ten coleoptiles were introduced into each test solution (2 mL) and incubated for 24 h at 22 °C in a roller-tube apparatus rotating at 0.25 rpm. Sections were then measured by using the images from a photographic enlarger (X3), and data were statistically analyzed (Kurtz et al., 1965).

## RESULTS AND DISCUSSION

The mass spectral data clearly shows that one, two, and three additional acetate groups have been introduced onto cytochalasin H (**2**) in **3**, **4** and **5**, respectively. Carbon-13 NMR spectra were run on **3–5** to confirm the location of the additional acetate groups and to allow comparison of the $^{13}C$ NMR spectra of **1–5** in order to confirm the assignment of the spectra. The $^{13}C$ NMR chemical shifts for **1–5** are given in Table I. Data for **1** and **2** have been reported previously (Cole et al., 1981) and are included here so that comparisons can be made for the entire series of compounds.

Table I. $^{13}C$ Chemical Shifts of Cytochalasin H and Its Acetate Derivatives[a]

| carbon | compound 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|
| 1 | 175.84 | 174.96 | 174.74 | 174.38 | 174.40 |
| 3 | 53.14 | 53.46 | 53.33 | 55.91 | 56.03 |
| 4 | 49.23 | 49.62 | 49.30 | 44.86 | 44.84 |
| 5 | 32.69 | 32.26 | 32.56 | 31.61 | 31.64 |
| 6 | 149.72 | 148.35 | 146.00 | 145.04 | 145.06 |
| 7 | 70.05 | 69.61 | 72.09 | 72.76 | 72.75 |
| 8 | 45.54 | 46.73 | 44.12 | 44.05 | 44.06 |
| 9 | 53.24 | 51.52 | 51.47 | 54.04 | 54.37 |
| 10 | 42.91 | 42.41 | 42.28 | 42.45 | 42.22 |
| 11 | 13.72 | 13.32 | 13.05 | 11.77 | 11.76 |
| 12 | 112.19 | 113.96 | 114.91 | 115.58 | 115.59 |
| 13 | 128.02 | 127.33 | 127.09 | 127.03 | 127.19 |
| 14 | 137.24 | 138.39 | 136.61 | 136.82 | 136.14 |
| 15 | 45.00 | 44.94 | 44.84 | 40.59 | 40.59 |
| 16 | 31.15 | 30.54 | 30.48 | 30.04 | 27.74 |
| 17 | 53.77 | 53.46 | 53.33 | 53.65 | 51.16 |
| 18 | 73.42 | 73.86 | 73.96 | 73.85 | 83.90 |
| 19 | 136.37 | 127.09 | 126.64 | 126.71 | 127.01 |
| 20 | 130.65 | 138.52 | 138.22 | 139.65 | 137.72 |
| 21 | 75.71 | 77.23 | 77.23 | 74.94 | 74.74 |
| 22 | 26.39 | 25.94 | 25.90 | 25.92 | 25.14 |
| 23 | 28.19 | 27.83 | 27.83 | 27.65 | 24.89 |
| 1′ | 137.53 | 137.44 | 137.38 | 136.94 | 137.01 |
| 2′ | 128.24 | 128.94 | 128.93 | 128.73 | 128.74 |
| 3′ | 129.24 | 129.29 | 129.39 | 130.16 | 130.18 |
| 4′ | 126.38 | 125.83 | 125.81 | 124.16 | 123.37 |
| N-2 acetate | | | | 25.33 | 25.33 |
| | | | | 171.11 | 171.05 |
| C-7 acetate | | | 20.57 | 20.59 | 20.60 |
| | | | 170.54 | 170.32 | 170.41 |
| C-18 acetate | | | | | 21.70 |
| | | | | | 169.96 |
| C-21 acetate | | 20.29 | 20.32 | 20.33 | 20.27 |
| | | 170.47 | 170.54 | 170.48 | 170.48 |

[a] In ppm downfield from $Me_4Si$.

The $^{13}C$ chemical shift of C-1 is easily assigned by comparison of **1–5** and remains relatively constant throughout the series. Similarly, the assignments of C-1′–C-6′ are straightforward based on intensity considerations, sford results, and consistency throughout the series **1–5**. The carbons in the exocyclic double bond, C-6 and C-12, were assigned on the basis of the sford results (singiet and triplet, respectively). the remaining $sp^2$ carbons were assigned from the shifts induced upon the introduction of acetate groups. Introduction of an acetate group on C-21 (**1** vs. **2**) should affect primarly the shifts of C-19 and C-20 while the shifts for C-13 and C-14 should remain relatively unchanged. Similarly, introduction of an acetate group on C-18 should also effect only the shifts of C-19 and C-20 but in the opposite direction from that of the acetate group on C-21. The assignments of C-13, C-14, C-19, and C-20 are consistent with these expectations.

For the alipahtic carbons, the assignments of the hydroxy-bearing carbons is relatively straightforward based on the sford results and the expected shift for the introduction of an acetate group. Comparison of the shifts in **1–3** confirm the assignments of C-7 and C-21 while the

Table II. $^1H$ Chemical Shifts of Cytochalasin H and Its Acetate Derivatives[a]

| proton | compound 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|
| 2 | 5.382 | 5.448 | 5.577 | | |
| 3 | 3.224 | 3.150 | 3.252 | 3.940 | 3.943 |
| 4 | 1.937 | 2.020 | 2.136 | 2.292 | 2.311 |
| 5 | 2.546 | 2.670 | 2.826 | 2.512 | 2.509 |
| 7 | 3.764 | 3.812 | 5.249 | 5.160 | 5.169 |
| 8 | 2.824 | 2.831 | 3.152 | 3.122 | 3.112 |
| 10a | 2.859 | 2.750 | 2.841 | 3.333 | 3.327 |
| 10b | 2.509 | 2.549 | 2.668 | 2.567 | 2.586 |
| 11 | 1.050 | 0.875 | 0.951 | 0.278 | 0.269 |
| 12a | 5.049 | 5.000 | 5.052 | 4.912 | 4.911 |
| 12b | 5.266 | 5.237 | 5.230 | 5.153 | 5.151 |
| 13 | 5.670 | 5.625 | 5.577 | 5.539 | 5.577 |
| 14 | 5.240 | 5.256 | 5.222 | 5.285 | 5.268 |
| 15a | 1.947 | 1.940 | 1.935 | 1.950 | 1.950 |
| 15b[b] | ~1.70 | ~1.74 | ~1.74 | ~1.75 | ~1.75 |
| 16[b] | ~1.70 | ~1.70 | ~1.74 | ~1.75 | ~1.75 |
| 17a | 1.839 | 1.768 | 1.897 | 2.150 | 2.076 |
| 17b | 1.496 | 1.459 | 1.531 | 1.570 | ~1.7 |
| 19 | 5.979 | 5.750 | 5.850 | 5.609 | 5.610 |
| 20 | 5.680 | 5.417 | 5.488 | 5.533 | 5.570 |
| 21 | 4.046 | 5.587 | 5.621 | 5.704 | 5.780 |
| 22 | 0.976 | 0.942 | 1.017 | 1.031 | 1.000 |
| 23 | 1.295 | 1.234 | 1.331 | 1.361 | 1.594 |
| 2'-6'[b] | 7.0-7.3 | 7.0-7.3 | 7.1-7.3 | 7.2-7.3 | 7.2-7.3 |
| Ac-C-7 | | | 1.935 | 1.906 | 1.907 |
| Ac-C-18 | | | | | 2.012 |
| Ac-C-21 | | 2.380 | 2.261 | 2.292 | 2.284 |
| Ac-N-2 | | | | 2.512 | 2.509 |

[a] In ppm downfield from $Me_4Si$. [b] Center of overlapped multiplet.

sford results give the assignment of C-18. Furthermore, it is clear that only in **5** is there an acetate group attached to C-18. The sford data confirm the assignment of C-9 as the remaining singlet. The assignment of the remainder of the methine carbons is based upon previous assignments of the $^{13}C$ NMR spectra of cytochalasins (Cole and Cox, 1981) and are consistent with the changes expected throughout the series **1–5**. For the methylene carbons, the chemical shift of C-10 should remain relatively unchanged throughout **1–5** and is assigned consistent with this expectation. Comparison with the assignment of the $^{13}C$ NMR spectra of other cytochalasins and shifts expected as a result of introduction of an acetate group led to the assignment of C-15 and C-17. The methyl carbons were assigned to be consistent with the assignments given previously for other cytochalasins.

Initial comparison of the $^1H$ NMR spectrum of **2** with that of **4** and **5** shows that one of the methyl doublets has undergone an upfield shift of ~0.7 ppm in **4** and **5**. Therefore, an analysis of the $^1H$ NMR spectra of **1–5** was carried out to confirm the assignment of the protons and to allow conformational diffeences among **1–5** to be determined. Several homonuclear decoupling experiments were carried out on **2** to establish assignments by using the proton–proton coupling constants. A two-dimensional $J$ correlated experiment (COSY) (Aue et al., 1976; Kumar et al., 1980) was also run on **2** to establish all of the coupling relationships. Analysis of this spectrum gave the chemical shift assignments for **2** given in Table II. The coupling constants given in Table III were obtained from a normal one-dimensional spectrum. The assignment of the proton chemical shifts of **1** and **3** (Table II) follows from that of **2** by considering the effect of an acetate group replacing a hydroxy group (Jackman and Sternhell, 1969). The assignment of the $^1H$ chemical shifts of **5** was also accomplished by using data from a two-dimensional $J$ correlated spectrum and homonuclear decoupling experiments. The assignment of **4** follows from that of **5**. It is clear from the $^1H$ spectra that the nitrogen in the five-membered ring is acetylated in both **4** and **5**.

Table III. Proton-Proton Coupling Constants for Cytochalasin H and Its Acetate Derivatives[a, b]

| coupling constant | compound 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|
| $J_{3,4}$ | 4.0 | 3.9 | 3.8 | 2.2 | 2.5 |
| $J_{3,10a}$ | 4.0 | 4.7 | 4.8 | 2.8 | 2.8 |
| $J_{3,10b}$ | 9.6 | 9.5 | 9.5 | 10.9 | 11.3 |
| $J_{4,5}$ | 4.9 | 4.9 | 4.9 | c | 3.8 |
| $J_{5,11}$ | 6.7 | 6.7 | 6.7 | 6.7 | 6.7 |
| $J_{7,8}$ | 10.7 | 10.8 | 11.5 | 10.4 | 10.3 |
| $J_{7,12}$ | 1.3 | 1.0 | 1.2 | 0.8 | 0.7, 1.5 |
| $J_{8,13}$ | 9.8 | 9.7 | 9.7 | 9.6 | 9.6 |
| $J_{10a,10b}$ | 13.5 | 13.4 | 13.4 | 12.5 | 12.6 |
| $J_{13,14}$ | 15.7 | 15.6 | 15.4 | 15.4 | 15.4 |
| $J_{14,15a}$ | 4.9 | 4.7 | 4.7 | 5.0 | 5.2 |
| $J_{14,15b}$ | 10.3 | 10.7 | 10.4 | 10.2 | 10.5 |
| $J_{16,22}$ | 6.5 | 6.2 | 6.2 | 6.2 | 6.6 |
| $J_{16,17a}$ | 2.9 | 2.8 | 2.9 | c | 3.7 |
| $J_{16,17b}$ | 3.0 | 2.1 | 2.3 | c | c |
| $J_{17a,17b}$ | 14.3 | 14.1 | 14.2 | c | 14.2 |
| $J_{19,20}$ | 16.8 | 17.2 | 16.6 | 16.5 | c |
| $J_{19,21}$ | 2.6 | 2.1 | 2.6 | 1.6 | c |
| $J_{20,21}$ | 2.3 | 2.2 | 2.3 | 1.3 | c |

[a] In hertz. [b] The following were not determined due to an overlap of peaks: $J_{15a,15b}$, $J_{15a,16}$, and $J_{15b,16}$. [c] Not determined due to an overlap of peaks.

Table IV. Percent Inhibition of Wheat Coleoptiles (*T. aestivum* L. cv. Wakeland), Relative to Controls, Obtained with Cytochalasins[a]

| compound | $10^{-3}$ M | $10^{-4}$ M | $10^{-5}$ M | $10^{-6}$ M | $10^{-7}$ M |
|---|---|---|---|---|---|
| cytochalasin A | 44 | 15 | 0 | 0 | |
| cytochalasin B | 65 | 62 | 0 | 0 | |
| cytochalasin E | 16 | 0 | 0 | 0 | |
| chaetoglobosin K | 100 | 84 | 62 | 43 | 14 |
| epoxycytochalasin H | 81 | 63 | 46 | 0 | |
| deacetylepoxycytochalasin H | 100 | 81 | 33 | 0 | |
| cytochalasin H (2) | 84 | 80 | 76 | 10 | |
| 1 | 100 | 82 | 40 | 16 | |
| 3 | 100 | 80 | 17 | 0 | |
| 4 | 51 | 39 | 0 | 0 | |
| 5 | 0 | 0 | 0 | 0 | |

[a] All numbers quoted represent significant inhibition: $P < 0.01$.

The solid-state structure of **2** has been reported previously (Beno et al., 1977) and shows that the five-membered ring is essentially planar whereas the six-membered ring has a boat conformation with C-5 and C-8 eclipsed. Consideration of the $^1H$ coupling constants (Table III) and the dihedral angle dependence of coupling constants suggests that **1–3** have a similar conformation in solution for the five- and six-membered ring as that found for **2** in the solid state. For example, in the solid state for **2** dihedral angles are estimated to be 120°, 50°, and 175°, respectively, for H-3–H-4, H-4–H-5, and H-7 and H-8 with coupling constants of 3.9, 4.9, and 10.8 Hz. However, it is clear that this does not hold for **4** and **5**. For example in comparison with **2**, $J_{3,4}$ decreases to 2.5 Hz, and $J_{4,5}$ decreases to 3.8 Hz whereas $J_{7,8}$ decreases only slightly to 10.3 Hz in **5**. In addition, the coupling constants $J_{3,10a}$ and $J_{3,10b}$ also change significantly between **2** and **5**. These data in addition to the upfield shift observed for the methyl protons on C-11 suggest that acetylation of the nitrogen in **4** and **5** produces steric crowding between the acetyl and phenyl groups resulting in a slight puckering of the five-membered ring in

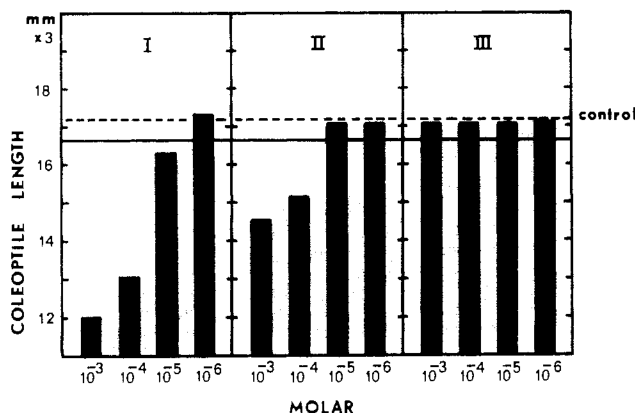
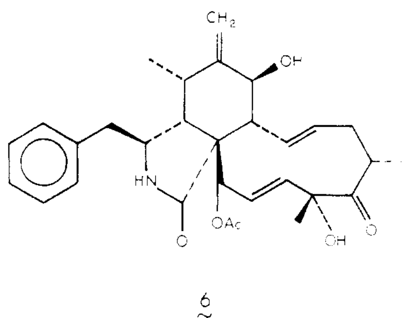



**Figure 1.** Growth-regulating activity of acetate derivatives of cytochalasin H (I = 3; II = 4; III = 5) in wheat coleoptile bioassays (*T. aestivum* L. cv. Wakeland). Control: dashed line. Inhibition is significant ($P < 0.01$) below the solid line.

4 and 5 and a shorter average distance between the phenyl ring and C-11. Consideration of the $^1H$ chemical shifts and proton–proton coupling constants and $^{13}C$ chemical shifts for the 11-membered ring suggests that the conformation of the 11-membered ring remains relatively constant throughout **1–5** (Beno et al., 1977).

Results for **1–5** and additional cytochalasins obtained from the wheat coleoptile bioassay are given in Table IV. For **3**, a significant inhibition ($P < 0.01$) was observed at $10^{-3}$, $10^{-4}$, and $10^{-5}$ M. Although somewhat less inhibitory, **4** was active at $10^{-3}$ and $10^{-4}$ M whereas **5** exhibited no inhibitory properties (Figure 1). Several cytochalasin D (**6**) derivatives have been examined for cytotoxic and an-




titumor activity (Minato et al., 1973). They concluded that the benzyl group at C-3 and the hydroxyl group at C-7 were essential for cytotoxic activity but the reduction of double bonds or acetylation of hydroxy groups in the macrocyclic ring did not substantially alter the cytotoxicity. It would appear from the data in Table IV and Figure 2 that the toxicity effects of cytochalasins and plant growth regulating effects are due to different modes of action. At $10^{-4}$ M, acetylation of the hydroxyl group on C-7 does not significantly alter the plant growth effects of cytochalasin H (Table IV, **2** vs. **3**), whereas acetylation of N-2 reduces the activity (**4**), and further acetylation of C-18 (**5**) results in an inactive compound (Figure 1). Thus, it would appear that the hydroxyl group at C-18, and to a lesser extent the NH group at N-2, is necessary for plant growth regulating effects. The $LD_{50}$ of a single oral dose of **1** and **2** in day-old chickens has been determined previously (Cole et al., 1981) to be 37.5 and 12.5 mg/kg, respectively. A single oral dose of **3** and **5** at a level of 200 mg/kg produced no visible adverse affects on day-old chickens. Thus, it appears that cytochalasin H behaves similar to cytochalasin D in that the 7-hydroxy group is necessary for toxicity effects in the chick bioassay. These results suggest different modes of action for toxicity effects and plant growth regulating effects. However, further studies are required before specific differences can be proposed.

**Registry No.** 1, 53760-20-6; **2**, 53760-19-3; **3**, 84499-89-8; **4**, 84499-90-1; **5**, 84499-91-2; cytochalasin A, 14110-64-6; cytochalasin B, 14930-96-2; cytochalasin E, 36011-19-5; chaetoglobosin K, 72509-61-6.

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