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Structure of a New [11]Cytochalasin, Cytochalasin H or Kodo-cytochalasin-1

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Abstract: The crystal structure determination of a new [11]cytochalasin toxin (kodo-cytochalasin-1 or cytochalasin H) has proven the constitution of this compound and provided new information on the conformation of the multiple ring structure in this class of fungal metabolites. The toxin, (7*S*,16*S*,18*S*,21*R*)-21-acetoxy-7,18-dihydroxy-16,18-dimethyl-10-phenyl[11]cytochalasa-6(12),13′,19′-trien-1-one, with the structure I, $C_{30}H_{39}NO_5$, is very similar to cytochalasin D, lacking a keto oxygen at C-17 in the 11-membered ring and having the opposite stereochemistry at C(18). The conformation of the multiple fused ring system is nearly identical with that of a *p*-bromobenzoate derivative of cytochalasin D, indicating that the fused ring system in these [11]cytochalasins is a fairly rigid structural unit. Strong hydrogen bonds involving the NH and C=O functions of the γ-lactam ring, present in all other cytochalasin structures, may be important in binding to the toxin's site of action. The toxin crystallizes from diethyl ether as stocky prisms with the monoclinic space group $P2_1$ and cell constants $a = 7.338\ (2)$ Å, $b = 13.053\ (6)$ Å, $c = 15.330\ (4)$ Å, $\beta = 97.02\ (2)°$, and $Z = 2$. The structure was solved by direct methods and refined by least squares to a final *R* factor of 0.049 for 2707 independent reflections. The ^1H and ^{13}C NMR spectra are also reported.

The toxic effects on a number of crop plants of a fungal metabolite isolated from a fungus (*Phomopsis* sp.) found infecting weevil damaged pecans were recently described. Tests with tobacco, wheat, and bean plants showed significant growth-inhibiting or toxic effects, with single doses as small as 10^{-4} mmol inhibiting floral development in tobacco. The compound was toxic, LD_{50} for day-old cockerels being but 12.5 mg/kg.[2] Preliminary chemical and spectroscopic analyses indicated that the compound was probably a new member of the class of fused polycyclic ring compounds, the cytochalasins. Although the effects of cytochalasin B on tip growth in plants have been described,[3] cytochalasins are best known for their singular and varied effects on animal cells. The most unusual of their properties is their ability to cause cells to extrude their nuclei,[4] leading to the formation of nuclei-free cells. At lower concentrations (ca. 1 μg/mL) cytochalasins interfere with cell division, not by preventing nuclear division but by preventing cytoplasmic division at the final stage by blocking the formation of contractile microfilament structures.[5] The result is binuclear or polynuclear cells.[6] Other effects of these toxins are clot retraction; inhibition of cytoplasmic or protoplasmic streaming, cardiac and smooth muscle contraction; interference with sugar uptake,[7] release of growth hormone,[8] platelet aggregation, nerve outgrowth, and thyroid secretion. Most of these effects are reversible, disappearing when the cells are flushed with toxin-free nutrient. Cytochalasins possess some antibiotic activity[9] and one, cytochalasin D, has been reported to be a selective antitumor agent.[10]

This structural and NMR study was undertaken to unambiguously determine the identity of the new toxin and to examine its relationship with the known cytochalasins.

Experimental Section

Colorless, irregular prismatic crystals of the toxin were grown from diethyl ether solution. Precession photographs indicated a monoclinic crystal system; the systematic absences ($0k0$ absent for $k = 2n + 1$) were consistent with space groups $P2_1$ and $P2_1/m$. $P2_1$ was chosen as the appropriate space group and this choice later confirmed by the intensity distribution statistics. The cell constants were determined by least-squares refinement of the setting angles of 15 well-centered reflections; they appear with other pertinent crystal data in Table I.

Three-dimensional intensity data were collected on an automated four-circle diffractometer using graphite monochromatized Mo Kα radiation. A total of 4531 intensities were measured, using the ω–2θ scan technique, for reflections having 2θ values between 4.0 and 50.0°. Of these, 2707 were independent and remained after averaging of multiply measured and symmetry related reflections. The scan rate for data collection was adjusted on the basis of intensity to give roughly constant relative accuracy for the measurements. Backgrounds were counted for a total of one-half the time spent in each scan, and the scan

Table I. Crystal Data

Cell constants [temperature, 21 (1) °C]
a = 7.338 (2) Å $\quad \alpha = \gamma = 90.00°$
b = 13.053 (6) Å $\quad \beta$ = 97.02 (2)°
c = 15.330 (4) Å
V = 1457.2 (9) Å^3
Space group, $P2_1$ (C_2^2, no. 4)
ρ_{obsd} = 1.128 (3) g/cm^3
ρ_{calcd} = 1.124 g/cm^3 for Z = 2
λ(Mo K$\bar{\alpha}$) = 0.71069 Å
μ(Mo K$\bar{\alpha}$) = 0.818 cm^{-1}
$e^{-\mu r_{min}}$ = 0.938
$e^{-\mu r_{max}}$ = 0.984
Intensity data 4.0° ≤ 2θ ≤ 50.0°
Independent reflections used in LS, 2707
Number for which $I \geq 3\sigma(I)$, 2022
Final $R(F)$, 0.049
Final $R(F^2)$, 0.079
Final GOF, 2.1
Data/parameter ratio, 8.6

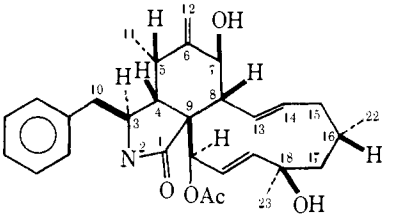
width was varied as a function of sin θ to compensate for α_1-α_2 splitting. Seven check reflections were monitored periodically; their intensities, used for a linear decay correction, decreased an average of 5% over the course of data collection. The data crystal was large, ca. 0.2 × 0.3 × 0.5 mm^3, but azimuthal scans for several reflections indicated that absorption effects were negligible compared to the normal scatter in the intensity data. The minimum and maximum transmission coefficients (Table I) dictated a maximum variation of 4.6% in the intensities and absorption corrections were accordingly deemed unnecessary. The estimated standard deviations in the reduced intensities (F^2) were calculated using $\sigma(F^2) = (r/Lp)[S + G^2(B_1 + B_2) + (pI)^2]^{1/2}$, where r is the scan rate, $1/Lp$ is the Lorentz-polarization correction derived for the monochromatized beam case,[11] S, B_1, and B_2 are the scan and background counts, G is the ratio of scan time to total background counting time, I is the net intensity, and p is a factor, chosen as 0.02, included in a term supposed to represent that component of the total error expected to be proportional to the diffracted beam intensity.[12]

The phase problem was solved by direct methods,[13] although not without considerable difficulty. The solution was found by using the programs MULTAN[14] and MAGIA[15] in conjunction. An initial structural fragment was extracted from an E map containing a false mirror, and this was slowly developed via a bootstrap procedure[16] to yield the entire molecule. Subsequent refinement, including location of the hydrogen atoms, was carried out by standard Fourier least-squares techniques. Most of these calculations were carried out using the CRYM crystallographic computing system.[17] Hydrogen atom contributions were included in the calculations but their parameters were not refined. All atoms heavier than hydrogen were refined anisotropically and an extinction correction[18] was included. The refinement was considered converged when no shifts in the final least-squares cycle exceeded 0.25 esd. The function minimized in the least-squares refinement was $\Sigma w(kF_o^2 - F_c^2)^2$. The final R factor was 0.049 [$Rw(F^2)$ = 0.079] and the "goodness-of-fit" was 2.1. The final difference map showed a few peaks of 0.25 e/Å^3 not particularly associated with the atomic coordinates. The atomic form factors for C, N, and O were taken from the International Tables,[19] while that for hydrogen was that given by Stewart et al.[20] The final values of the refined parameters and their standard deviations estimated from the diagonal elements of the inverted matrix from the final least-squares cycle are given in Table II (see paragraph at end of paper regarding supplementary material). The absolute configuration of this compound was not determined in this study; the correct enantiomer was chosen by analogy with the structures of the three cytochalasins for which the absolute stereochemistry is known.[21-23]

Proton NMR spectra were obtained on a Varian HA-100 spectrometer operating in the frequency sweep mode with a probe temperature of 30 °C. A sweep width of 1000 Hz and a sweep time of 500 s were used. Proton decoupled, natural abundance ^{13}C spectra were obtained on a JEOL PFT-100 spectrometer with the EC-100 data system. A pulse angle of 45° was used with a repetition time of 3 s. The spectral width utilized was 5000 Hz with a total of 8192 data points. The carbon-13 spectrum consists of 27 peaks. Off-resonance decoupling experiments show that the peak at 53.793 ppm is due to two overlapping resonances. The intensities of the peaks at 128.900 and 129.095 ppm suggest that they are due to two carbons each and are typical for the ortho and meta carbons of a monosubstituted benzene ring. The carbon resonances were assigned (Table III) on the basis of off-resonance and gated decoupling experiments, along with known chemical shift correlations. These assignments are in agreement with those for cytochalasins B and D.[24]

Results

The toxin has the structural formula I and the full systematic name[25] given in the abstract. In a preliminary communication,[26] we assigned the trivial name cytochalasin H to this compound, but now note that Pendse[27] isolated a toxin from *Phomopsis paspalli* which was deduced on the basis of spectrochemical information to have the gross structure I by Pat-



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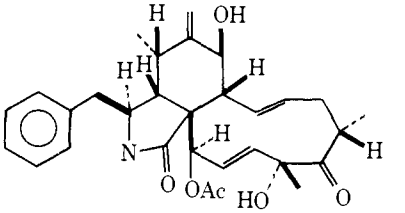


II

wardhan et al.[28] and for which they proposed the name kodo-cytochalasin-1. Comparison of the infrared and mass spectra data of Wells et al.[2] for cytochalasin H (henceforth abbreviated CytH) and our NMR results (Table III) with those reported by Patwardhan and co-workers demonstrated that the compounds are identical; this has been recently confirmed by an independent structure determination by McMillan and co-workers[29] of an authentic sample of kodo-cytochalasin-1.

CytH is most closely related to cytochalasin D (CytD), II, which differs from it by possessing a keto oxygen at C(17) and by having the opposite stereochemistry at C(18).

Figure 1 is a stereodiagram of the CytH molecule, showing in particular the characteristic configuration of the two smaller fused rings. The lactam portion of the five-membered ring is structurally very similar to a cis-peptide linkage; this is understandable as N(2), C(3), C(4) and the alkyl-phenyl moiety have been inferred to be derived from a precursor phenylalanine molecule.[31] The six-membered ring has a boat conformation as a consequence of C(6) having sp^2 geometry.[32] C(5) and C(8) are constrained to be eclipsed by the fusion to the γ-lactam ring at C(4)-C(9). The fusion of the 11-membered ring at C(8) and C(9) undoubtedly contributes to the overall strain of the molecule that has resulted in the C(4)-C(9) bond being somewhat lengthened from a normal single bond value of 1.541 (3) Å.[33] Other than the C(4)-C(9) bond, all other bond distances (Table IV) in the structure are within expected values considering the local environments of attached groups.

There are three well-localized C-C double bonds in the structure: the two *trans*-ethylene groups at C(13) and C(19) and the methide, C(6)-C(12). The former are oriented roughly parallel to each other (Figure 1), about 3.6 Å apart. The planes of the two groups, however, are inclined to each other by a

Table II. Final Least-Squares Parameter Values[a]

Atom	X	Y	Z	U_{11}	U_{22}	U_{33}	U_{12}	U_{13}	U_{23}
C(1)	394 (4)	1590 (3)	2014 (2)	42 (2)	50 (2)	46 (1)	−8 (2)	5 (1)	−2 (1)
N(2)	−509 (3)	1790 (2)	2694 (1)	38 (1)	79 (2)	50 (1)	14 (1)	8 (1)	7 (1)
C(3)	262 (4)	1382 (3)	3541 (2)	39 (2)	76 (2)	45 (2)	4 (2)	5 (1)	7 (2)
C(4)	2122 (4)	930 (3)	3356 (2)	39 (2)	64 (2)	41 (1)	4 (2)	5 (1)	2 (1)
C(5)	3775 (4)	1502 (3)	3849 (2)	39 (2)	90 (2)	48 (2)	−0 (2)	4 (1)	−7 (2)
C(6)	3891 (4)	2540 (3)	3468 (2)	45 (2)	80 (2)	64 (2)	−14 (2)	20 (2)	−22 (2)
C(7)	4137 (4)	2555 (3)	2497 (2)	39 (2)	55 (2)	58 (2)	−0 (2)	5 (1)	−7 (1)
C(8)	3874 (4)	1494 (2)	2069 (2)	40 (2)	47 (2)	45 (1)	2 (1)	6 (1)	−3 (1)
C(9)	2118 (4)	970 (3)	2329 (2)	38 (2)	52 (2)	39 (1)	0 (1)	4 (1)	−0 (1)
C(10)	−1050 (4)	596 (4)	3861 (2)	36 (2)	113 (3)	65 (2)	−2 (2)	5 (2)	23 (2)
C(11)	3829 (4)	1448 (4)	4859 (2)	58 (2)	148 (4)	49 (2)	−15 (2)	−0 (2)	−9 (2)
C(12)	3769 (6)	3417 (4)	3884 (2)	131 (4)	95 (3)	97 (3)	−42 (3)	68 (3)	−44 (2)
C(13)	3976 (4)	1614 (3)	1094 (2)	52 (2)	45 (2)	50 (1)	−10 (2)	8 (1)	0 (1)
C(14)	5250 (4)	1185 (3)	690 (2)	51 (2)	64 (2)	52 (2)	−14 (2)	12 (1)	−11 (2)
C(15)	5399 (4)	1320 (3)	−281 (2)	75 (2)	64 (2)	58 (2)	−15 (2)	23 (2)	−9 (2)
C(16)	4704 (4)	391 (3)	−848 (2)	71 (2)	54 (2)	49 (2)	−1 (2)	14 (2)	−1 (2)
C(17)	2589 (4)	297 (3)	−919 (2)	74 (2)	54 (2)	49 (2)	62 (2)	0 (2)	−1 (2)
C(18)	1797 (4)	−672 (3)	−542 (2)	50 (2)	56 (2)	59 (2)	2 (2)	5 (1)	−18 (2)
C(19)	2305 (4)	−705 (3)	445 (2)	52 (2)	46 (2)	56 (2)	−2 (2)	2 (1)	−3 (2)
C(20)	1370 (4)	−243 (3)	1004 (2)	48 (2)	55 (2)	51 (2)	−3 (2)	5 (1)	−9 (1)
C(21)	1867 (4)	−141 (3)	1972 (2)	52 (2)	49 (2)	57 (2)	−3 (2)	7 (1)	−2 (2)
C(22)	5295 (6)	496 (4)	−1762 (2)	104 (3)	77 (2)	53 (2)	33 (2)	19 (2)	−3 (2)
C(23)	−278 (5)	−696 (4)	−805 (2)	56 (2)	100 (3)	68 (2)	10 (2)	−7 (2)	−32 (2)
C(24)	3569 (7)	−1509 (3)	2751 (2)	162 (4)	49 (2)	73 (2)	11 (3)	5 (3)	8 (2)
C(25)	5541 (10)	−1878 (4)	2980 (4)	220 (6)	64 (3)	120 (3)	44 (4)	−53 (4)	9 (2)
C(26)	−434 (4)	229 (3)	4792 (2)	47 (2)	99 (3)	60 (2)	−9 (2)	12 (2)	21 (2)
C(27)	−712 (5)	829 (3)	5504 (2)	77 (2)	101 (3)	73 (2)	14 (2)	27 (2)	17 (2)
C(28)	−33 (6)	513 (4)	6350 (2)	109 (3)	117 (3)	62 (2)	−7 (3)	18 (2)	−8 (2)
C(29)	921 (6)	−387 (4)	6476 (2)	112 (3)	108 (4)	69 (2)	2 (3)	4 (2)	24 (2)
C(30)	1218 (5)	−984 (3)	5779 (2)	78 (3)	93 (3)	86 (2)	1 (2)	8 (2)	29 (2)
O(1)	−84 (3)	1860 (3)	1250 (1)	54 (1)	65 (1)	43 (1)	7 (1)	1 (1)	5 (1)
O(2)	5980 (2)	2910 (2)	2437 (1)	41 (1)	51 (1)	59 (1)	−6 (1)	4 (1)	−0 (1)
O(3)	2626 (3)	−1532 (2)	−928 (1)	65 (1)	51 (1)	74 (1)	−5 (1)	18 (1)	−21 (1)
O(4)	3585 (3)	−661 (2)	2253 (1)	80 (2)	49 (1)	62 (1)	10 (1)	−1 (1)	9 (1)
O(5)	2212 (5)	−1876 (3)	2960 (2)	216 (4)	82 (2)	169 (3)	−12 (3)	71 (3)	50 (2)

	X	Y	Z		X	Y	Z		X	Y	Z
H(1)	−1558	2159	2630	H(14)	3565	3418	4577	H(27)	4778	−232	−2147
H(2)	6019	3000	1752	H(15)	3875	4138	3537	H(28)	4770	1033	−2065
H(3)	2047	−2133	−947	H(16)	2937	2081	709	H(29)	−584	−865	−1475
H(4)	459	1948	4068	H(17)	6263	704	1073	H(30)	−994	−1466	−591
H(5)	2276	146	3599	H(18)	6840	1447	−359	H(31)	−894	−101	−663
H(6)	5044	1108	3748	H(19)	4598	1990	−514	H(32)	5316	−2509	3104
H(7)	3105	3054	2147	H(20)	5297	−299	−534	H(33)	6398	−1595	2273
H(8)	4964	964	2310	H(21)	2055	333	−1613	H(34)	6031	−1483	3422
H(9)	−1126	−63	3421	H(22)	2083	949	−575	H(35)	−1451	1556	5406
H(10)	−2404	943	3842	H(23)	3519	−1138	705	H(36)	−256	984	6912
H(11)	2772	1904	4997	H(24)	75	109	729	H(37)	1426	−626	7144
H(12)	4947	1734	5151	H(25)	690	−475	2237	H(38)	2010	−1685	5886
H(13)	3634	713	5038	H(26)	6425	547	−1743	H(39)	721	−1171	4374

[a] Positional parameters have been multiplied by 10^4 and the U_{ij} elements have been multiplied by 10^3. The form of the anisotropic temperature factor is $\exp[-2\pi^2(U_{11}h^2a^{*2} + \cdots + 2U_{23}klb^*c^*)]$. All of the hydrogen atoms were assigned $B = 6.0$ Å^2. Estimated standard deviations in this and the following tables are given in parentheses.

substantial angle due to the constraints imposed by the 11-membered ring being closed.

The phenyl group is planar well within experimental error. The bond C(10)–C(26), however, makes a 4° angle with the least-squares plane through the phenyl ring, apparently caused by packing forces. The five-membered lactam ring is slightly but significantly puckered toward the six-membered ring.

All three of the possible hydrogen-bond donors form hydrogen bonds in the crystal. Table V lists the hydrogen bonds and other intermolecular van der Waals contacts. The oxygen atoms of the acetate ester moiety, which should by reason of exposure be likely candidates as acceptors, are completely uninvolved. The acetate group as a consequence has substantially higher thermal motion than any other part of the molecule and librates about O(4) in a cavity produced by the packing. The NH and C=O groups of the γ-lactam ring are respectively a donor and an acceptor of moderately strong hydrogen bonds with hydroxyl groups O(2) and O(3) of neighboring molecules. O(2) is in turn hydrogen bonded to O(3) of a molecule related by a crystallographic twofold screw operation. This ring of three hydrogen bonds (Figure 2) links molecules along the a axis and along the b axis. Only van der Waals forces hold the molecules together in the c-axis direction.

The general thermal motion of the molecule is apparent in the ORTEP[30] Figures 1 and 2. The amplitudes of vibration are generally in the range 0.18–0.30 Å. The atoms of the acetate ester have vibration amplitudes in the range 0.25–0.50 Å. None

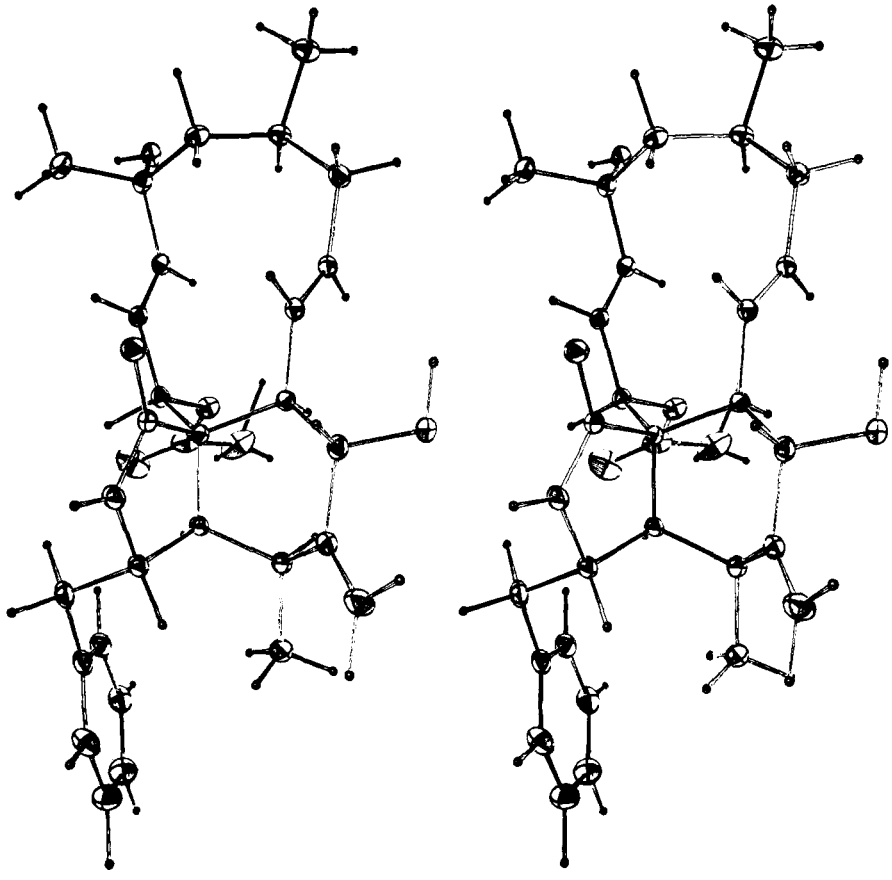


Figure 1. ORTEP stereodiagram of the cytochalasin H toxin. Thermal ellipsoids (except for hydrogen atoms) are shown at the 10% probability level.

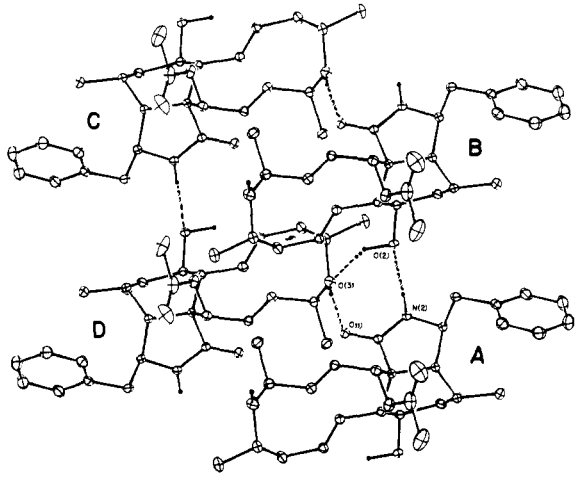


Figure 2. Hydrogen bonds (dashed lines) in the cytochalasin H crystal structure. Molecule A is at coordinates x,y,z as given in Table II. Molecules B, C, and D are respectively at $1+x,y,z$, $-x,\frac{1}{2}+y,z$, and $-1-x,\frac{1}{2}+y,z$.

of the bond distances have been corrected for thermal motion.

Discussion and Comparison of Cytochalasin Structures

The structures of five other cytochalasins have been reported: cytochalasin B (Ag^+ adduct),[21] cytochalasin D′ (where R = *p*-bromobenzoate),[22] cytochalasin E (Ag^+ adduct),[23] cytochalasin G,[34] and chaetoglobosin.[35]

The common features possessed by the cytochalasins are (1) the central isoindole ring structure of common stereochemistry, (2) the large macrocyclic ring fused to the isoindole ring at C(8) and C(9), and (3) an aromatic substituent, either a phenyl

Table III. Peak Assignments of the NMR Spectra

^{1}H assignments			^{13}C assignments		
Proton type	Peak	ppm	C		ppm
CH_3 (C_{23})	Singlet	1.32	1	C	174.400
CH_3 (C_{11})	Br doublet	1.03	3	CH	53.793
CH_3 (C_{22})	Sharp doublet	0.91	4	CH	50.281
CH_3 (C_{25})	Singlet	2.22	5	CH	32.870
			6	C	148.212
	Multiplet	1.84	7	CH	69.838
	Multiplet	2.16	8	CH	47.258
			9	C	51.939
	AB quartet	2.78	10	CH_2	45.550
			11	CH_3	14.045
	Multiplet	3.22	12	CH_2	113.828
	Doublet	3.80	13	CH	[127.242][a]
			14	CH	[127.047][a]
Vinyl type	Br singlet	5.06	15	CH_2	42.868
			16	CH	31.163
Vinyl type	Br singlet	5.30	17	CH_2	53.793
Vinyl type	Br singlet	5.94	18	C	74.180
			19	CH	[138.264][b]
Vinyl type	Multiplet	5.64	20	CH	[138.505][b]
Vinyl type	Multiplet	5.80	21	CH	77.543
Vinyl type	Multiplet	5.46	22	CH_3	26.482
			23	CH_3	28.481
Aromatic	Multiplet	7.18	24	C	170.014
protons	centered at		25	CH_3	20.826
			26	C	137.389
			27, 31	CH	129.095
			28, 30	CH	128.900
			29	CH	125.876

[a,b] The assignments of atoms in these groups are possibly reversed or exchanged; further data on similar compounds are needed if unambiguous assignments of these atoms are to be made.

Table IV. Bond Distances (Å) and Angles (deg)[a]

C(1)-N(2)	1.327 (4)	C(7)-O(2)	1.444 (4)	C(18)-C(19)	1.514 (4)
C(1)-O(1)	1.233 (3)	C(8)-C(9)	1.554 (4)	C(19)-C(20)	1.308 (4)
C(1)-C(9)	1.529 (4)	C(8)-C(13)	1.514 (4)	C(20)-C(21)	1.491 (4)
N(2)-C(3)	1.453 (4)	C(9)-C(21)	1.554 (4)	C(21)-O(4)	1.450 (4)
C(3)-C(4)	1.544 (4)	C(10)-C(26)	1.522 (5)	O(4)-C(24)	1.345 (5)
C(3)-C(10)	1.528 (5)	C(13)-C(14)	1.308 (4)	C(24)-C(25)	1.525 (7)
C(4)-C(5)	1.541 (4)	C(14)-C(15)	1.517 (5)	C(24)-O(5)	1.184 (6)
C(4)-C(9)	1.575 (4)	C(15)-C(16)	1.543 (4)	C(26)-C(27)	1.378 (5)
C(5)-C(6)	1.482 (4)	C(16)-C(17)	1.547 (4)	C(27)-C(28)	1.394 (5)
C(5)-C(11)	1.546 (5)	C(16)-C(22)	1.523 (5)	C(28)-C(29)	1.370 (6)
C(6)-C(7)	1.522 (4)	C(17)-C(18)	1.534 (5)	C(29)-C(30)	1.361 (5)
C(6)-C(12)	1.319 (5)	C(18)-C(23)	1.527 (5)	C(30)-C(31)	1.392 (5)
C(7)-C(8)	1.535 (4)	C(18)-O(3)	1.438 (4)	C(31)-C(26)	1.389 (5)

O(1)-C(1)-C(9)	125.4	C(7)-C(8)-C(13)	108.1	C(19)-C(18)-O(3)	108.9
O(1)-C(1)-N(2)	125.4	C(9)-C(8)-C(13)	116.2	O(3)-C(18)-C(23)	109.5
N(2)-C(1)-C(9)	109.2	C(1)-C(9)-C(4)	103.5	C(18)-C(19)-C(20)	123.8
C(1)-N(2)-C(3)	116.9	C(1)-C(9)-C(8)	111.5	C(19)-C(20)-C(21)	127.5
N(2)-C(3)-C(4)	103.4	C(1)-C(9)-C(21)	108.9	C(9)-C(21)-C(20)	116.0
N(2)-C(3)-C(10)	109.9	C(4)-C(9)-C(8)	111.8	C(9)-C(21)-O(4)	105.7
C(4)-C(3)-C(10)	114.1	C(4)-C(9)-C(21)	107.9	O(4)-C(21)-C(20)	110.6
C(3)-C(4)-C(5)	112.7	C(8)-C(9)-C(4)	112.8	C(21)-O(4)-C(24)	119.0
C(3)-C(4)-C(9)	106.1	C(3)-C(10)-C(26)	112.7	O(4)-C(24)-C(25)	108.4
C(5)-C(4)-C(9)	112.3	C(8)-C(13)-C(14)	123.1	O(4)-C(24)-O(5)	123.5
C(4)-C(5)-C(6)	109.2	C(13)-C(14)-C(15)	123.7	O(5)-C(24)-C(25)	128.1
C(4)-C(5)-C(11)	113.1	C(14)-C(15)-C(16)	113.8	Phenyl group	
C(6)-C(5)-C(11)	116.2	C(15)-C(16)-C(17)	111.2	C(10)-C(26)-C(27)	120.6
C(5)-C(6)-C(7)	114.6	C(15)-C(16)-C(22)	109.6	C(10)-C(26)-C(31)	120.1
C(5)-C(6)-C(12)	126.4	C(17)-C(16)-C(22)	109.8	C(27)-C(26)-C(31)	119.2
C(7)-C(6)-C(12)	119.0	C(16)-C(17)-C(18)	117.5	C(26)-C(27)-C(28)	119.9
C(6)-C(7)-C(8)	112.5	C(17)-C(18)-C(19)	110.0	C(27)-C(28)-C(29)	120.2
C(6)-C(7)-O(2)	106.9	C(17)-C(18)-C(23)	109.2	C(28)-C(29)-C(30)	120.6
O(2)-C(7)-C(8)	109.4	C(17)-C(18)-O(3)	106.8	C(29)-C(30)-C(31)	119.9
C(7)-C(8)-C(9)	110.6	C(19)-C(18)-C(23)	112.2	C(30)-C(31)-C(26)	120.2

[a] The estimated standard deviations in the angles are generally in the range 0.3–0.5°.

Table V. Hydrogen Bonds and van der Waals Contacts[a]

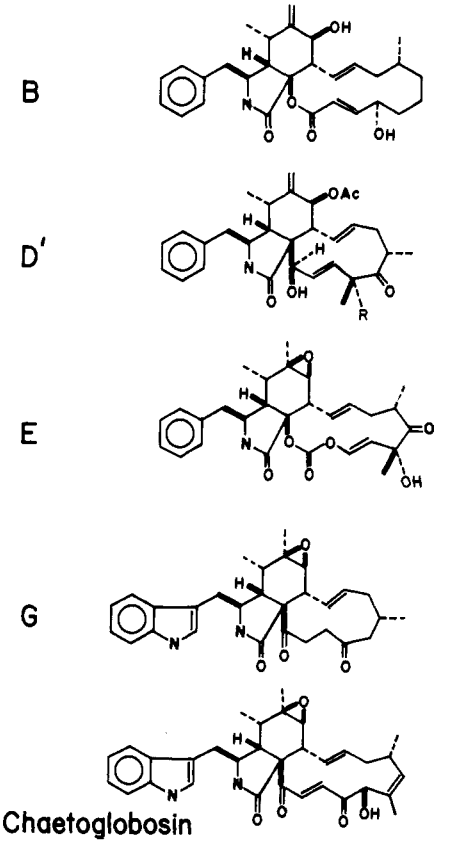
Hydrogen bonds Donor	Acceptor	Distances, Å D-H	H···A	D-A	Angle, deg, D-H-A
N(2)-H(1)···O(2)[a]		0.90	2.04	2.946	176
O(3)[b]-H(3)[b]···O(1)		0.89	1.96	2.811	158
O(2)[a]-H(2)[a]···O(3)[b]		1.06	1.81	2.740	144

Close intermolecular approaches

Atom	Distance, Å
H(6)[c]···H(10)	1.87
H(21)[d]···H(37)	2.28
H(30)[b]···H(16)	2.36
H(20)[b]···H(7)	2.45
H(27)[e]···H(2)	2.47
H(28)[f]···H(34)	2.48
O(2)[f]···H(27)	2.51
O(1)[g]···H(30)	2.57

[a] Symmetry relations: a, $1 + x,y,z$; b, $-x,\frac{1}{2} + y,-z$; c, $x - 1,y,z$; d, $x,y,1 + z$; e, $1 - x,\frac{1}{2} - y,-z$; f, $1 - x,y - \frac{1}{2},-z$; g, $-x,y - \frac{1}{2},-z$.

or indole, at C(10). The cytochalasan core has several substituents, usually including a methyl group at C(5), a methyl or methide at C(6), and either a hydroxyl group at C(7) or an epoxide bridging C(6)-C(7). The five-membered γ-lactam ring is an invariant feature. The large macrocyclic ring is quite variable, both in size and in the number and type of substituent groups.



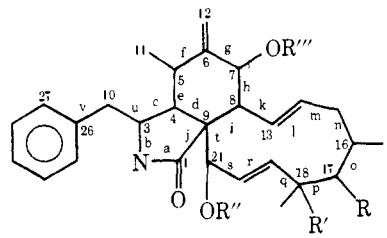
The bond distances of the invariant cytochalasan core for the structures whose coordinates are available are given in

Table VI. Comparison of Bond Distances (Å) in the Cytochalasan Core of Five Cytochalasin Structures

	Cytochalasin				
	B (phomin)	E	G	D′	H
C(1)-N(2)	1.34 (9)	1.376 (5)	1.38 (1)	1.37 (2)	1.327 (4)
C(1)-O(1)	1.26 (9)	1.24 (5)	1.20 (1)	1.19 (2)	1.233 (3)
N(2)-C(3)	1.40 (9)	1.51 (5)	1.45 (1)	1.41 (2)	1.453 (4)
C(3)-C(10)	1.72 (9)	1.48 (5)	1.53 (2)	1.55 (2)	1.528 (5)
C(3)-C(4)	1.57 (9)	1.55 (5)	1.57 (1)	1.54 (2)	1.544 (4)
C(4)-C(5)	1.63 (9)	1.55 (5)	1.56 (1)	1.56 (2)	1.541 (4)
C(4)-C(9)	1.49 (9)	1.59 (5)	1.56 (1)	1.54 (2)	1.575 (4)
C(5)-C(11)	1.65 (9)	1.59 (5)	1.54 (2)	1.51 (2)	1.546 (5)
C(5)-C(6)	1.40 (9)	1.602 (5)	1.51 (2)	1.52 (2)	1.482 (4)
C(6)-C(12)	1.35 (9)	1.46 (5)[a]	1.50 (2)[a]	1.29 (2)	1.319 (5)
C(6)-C(7)	1.44 (9)	1.47 (5)	1.46 (1)	1.52 (2)	1.522 (4)
C(6)-O(2)		1.48 (5)[b]	1.47 (1)[b]		
C(7)-O(2)	1.51 (9)	1.47 (5)[b]	1.45 (1)[b]	1.41 (2)	1.444 (4)
C(7)-C(8)	1.52 (9)	1.56 (5)	1.52 (1)	1.57 (2)	1.535 (4)
C(8)-C(9)	1.61 (9)	1.58 (5)	1.60 (1)	1.57 (2)	1.554 (4)
C(8)-C(13)	1.54 (9)	1.57 (5)	1.50 (1)	1.48 (2)	1.514 (4)
C(9)-C(1)	1.49 (9)	1.55 (5)	1.57 (1)	1.56 (2)	1.529 (4)
C(9)-C(21)			1.53 (1)	1.55 (2)	1.554 (4)
C(9)-O	1.51 (9)	1.41 (5)			

[a] Methyl group. [b] Epoxide.

Table VII. Selected Torsion Angles in Cytochalasin Structures[a]



		Cytochalasin				
Angle	Defining atoms	B	E	G	D	H
a	C(9)–C(1)–N(2)–C(3)	3.4	−19.3	−5.6	4.3	−1.3
b	C(1)–N(2)–C(3)–C(4)	−2.5	21.7	13.9	4.8	6.8
c	N(2)–C(3)–C(4)–C(5)	115.0	109.1	106.0	111.7	114.2
d	C(3)–C(4)–C(9)–C(8)	125.1	129.9	130.3	133.3	128.5
e	C(3)–C(4)–C(5)–C(6)	−68.7	−66.8	−70.9	−70.0	−69.2
f	C(4)–C(5)–C(6)–C(7)	−58.8	−57.3	−54.7	−63.4	−59.9
g	C(5)–C(6)–C(7)–C(8)	11.7	3.1	2.7	16.2	10.2
h	C(6)–C(7)–C(8)–C(9)	44.1	50.6	52.7	42.6	46.7
i	C(7)–C(8)–C(9)–C(1)	65.2	69.0	58.3	59.8	61.5
j	N(2)–C(1)–C(9)–C(4)	−2.8	9.2	−5.4	−11.6	−4.7
k	C(7)–C(8)–C(13)–C(14)	136.2	134.1	131.0	114.0	116.8
l	C(8)–C(13)–C(14)–C(15)	171.1	180.0	181.3	177.7	181.6
m	C(13)–C(14)–C(15)–C(16)	−129.1	−127.2	−124.8	−91.2	−103.1
n	C(14)–C(15)–C(16)–C(17)	76.6	68.2	55.5	73.7	71.0
o	C(15)–C(16)–C(17)–C(18)	−165.2	−131.6	−91.0	−131.1	−117.6
p	C(16)–C(17)–C(18)–C(19)	172.7	66.3	151.2	66.1	65.3
q	C(17)–C(18)–C(19)–C(20)	−101.4	134.7	−67.1	76.0	84.8
r	C(18)–C(19)–C(20)–C(21)			−75.0	−175.0	−173.1
s	C(19)–C(20)–C(21)–C(9)			165.9	136.5	118.9
t	C(20)–C(21)–C(9)–C(1)			−19.5	−52.5	−53.8
u	C(4)–C(3)–C(10)–C(26)	51.4	−64.2	−60.6	162.2	−71.1
v	C(3)–C(10)–C(26)–C(27)	88.4	107.2	94.8	86.3	101.9

[a] Lower case letters refer to the torsion angles (degrees) of the indicated bonds. The figure and numbering scheme are for CytH (R = H_2; R′ = OH; R″ = Ac; R‴ = H) and CytD′ (R = O; R′ = *p*-$BrC_6H_4CO_2$—; R″ = H).

Table VI. The agreement for each of the bonds is generally within one standard deviation, and all the distances shown are within three standard deviations of the weighted mean. Unfortunately, the lack of precision of the other structures prevents independent verification of the long C(4)–C(9) bond we observe in CytH. Comparison of the torsion angles, Table VII, shows that the conformations of the fused five- and six-membered rings in these molecules are very close, and more surprisingly a portion of the large ring, starting at C(8) and running to C(18) of C(19) (except for CytG), has a fairly constant conformation in spite of the different sizes and expected flexibility of these rings. Because the cumulative effect of small

differences in torsion angles (as one goes down the chain) can result in large differences in the absolute shape of the molecule, a more convenient comparison is afforded by superimposing ORTEP[30] diagrams of the molecules. We have calculated such overlapping diagrams for all the structures for which we have coordinates and find that in each case the cytochalasan core can be very nearly perfectly superimposed on the others. The differences, though apparent, are very slight, involving small changes in the conformation of the six-membered ring which arise either from formation of the epoxide at C(6)–C(7) or from the strain induced by the large macrocyclic ring. We give in Figure 3 one such overlap diagram, showing CytH superimposed on cytochalasin D′.

Because of the variation in the size and composition of the largest ring of the different cytochalasins, and the constraints imposed on several of them by complexation with Ag^+ ions, these rings are not expected to overlap well when the structures are superimposed. Even rings of the same size would not be expected to have the same conformation because of the number of methylene linkages in the ring about which free rotation could occur. It was consequently surprising to find the overlap agreement between CytH and CytD′ (Figure 3) to be quite good (cf. Table VII). In spite of some differences in the composition [e.g., CytD′ possesses a keto oxygen at C(17)] and differences in the number and size of attached substituents [CytH has an acetate ester at C(21), CytD′ a *p*-bromobenzoate ester at C(18)], the 11-membered rings of these molecules overlap extremely well. We conclude from this that the 11-membered ring is in a preferred conformation. Furthermore, as the peripheral groups (*p*-bromobenzoate, benzyl and acetate ester) are in different orientations in their respective crystal structures, the packing forces in the two crystal structures must be very different, and this perferred conformation of the 11-membered ring must be quite stable. In other words, the entire fused ring framework of cytochalasins D and H, excluding the peripheral substituents, is a stable, relatively rigid structural unit; we expect the ring framework of these molecules to maintain the pictured solid-state conformation even in solution.

Minato and co-workers[36] have examined a large number of CytD derivatives for cytotoxic and antitumor activity. They conclude that the large macrocyclic ring is required to be intact but that modifications of the ring, such as reduction of double bonds or acylation of hydroxo groups, do not substantially alter the cytotoxicity. The benzyl group at C(3) and the hydroxyl group at C(7), however, are essential for cytotoxic activity. The exact nature of the cytotoxicity was not indicated and is probably due to a combination of different physiological effects of the toxin. Their results do indicate, however, that the toxic nature of the cytochalasins should not vary considerably with changes in the large macrocyclic ring—consistent with the similar biological effects of the members of this class of toxins. The invariance of the conformation of the central core structure thus assumes major importance in cytochalasin toxicity; the configuration of the core and the direct substituents on the core are important, while the large macrocycle seems to be essential only in its bulk. We consequently speculate that the large variable ring provides at most some type of shielding function when the toxin binds to its site of action, and that the core of the molecule, with the rigidly positioned C=O, NH, and C(7)–OH groups functioning as principal binding agents. Further biological studies, with a much more restrictive assay than general toxicity, are required before a specific model of binding of the toxin can be formulated.

Finally, we wish to note a startling resemblance between the core of the cytochalasins and several monoterpenoid compounds of insect origin, the iridomyrmecins, III, which possess insecticidal activity.[37]

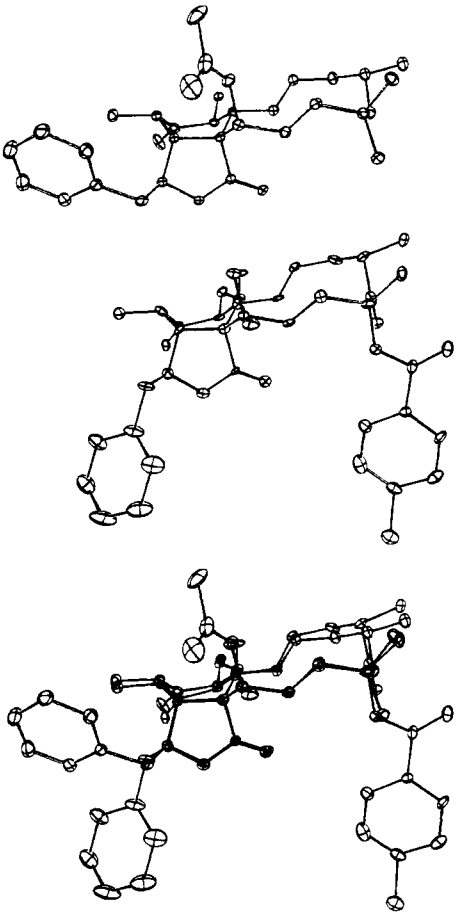
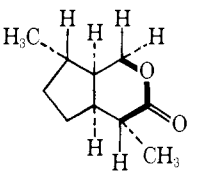


Figure 3. Superposition of ORTEP diagrams for cytochalasins D′ and H: (top) cytochalasin H; (middle) cytochalasin D′ (*p*-bromobenzoate ester of cytochalasin D); (bottom) superimposed diagrams.



III

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Supplementary Material Available: Listing of structure factor amplitudes (22 pages). Ordering information is given on any current masthead page.

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Laser Raman Scattering from an Enzyme of Well-Documented Structure, Human Carbonic Anhydrase B

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Abstract: Raman spectra are reported for human carbonic anhydrase B and their interpretation compared with the structure determined by x-ray diffraction. The Raman spectra provide quite satisfactory quantitative agreement with x-ray data. For example, Raman reveals 19% helix, 39% β structure, and 42% "disordered", compared with x-ray values of 17, 40, and 43%, respectively. X-ray-derived models show four tyrosines "buried" and four "exposed", while quantitative treatment of the Raman data provides values of 3.7 "buried" and 4.3 "exposed". The Phe/Tyr ratio in human carbonic anhydrase B is determined as 1.41 by Raman and 1.38 by x ray. The Raman spectra indicate a conformation for human carbonic anhydrase B's two methionine residues in which C_α is trans to S and C_β gauche to the methionyl methyl. This information is not available from x-ray diffraction. Conversion of the native protein to the apo form is accompanied by little or no spectroscopically observable conformational change.

Early development of assignments of laser Raman spectra of proteins derived largely from pioneering studies by Lord and Yu[2] of proteins of well-documented structure. It is now apparent that protein Raman spectra are rich in structural information.[3,4] As the technique is now being widely applied to an increasing number of proteins, it is worthwhile to again examine a protein of well-documented structure as a test of the validity of recent premises underlying the interpretation of protein structure by Raman scattering.

As our model, we have chosen human carbonic anhydrase B (HCAB) (E.C. 4.2.1., carbonate hydrolyase), a zinc metalloenzyme which catalyses the reversible hydration of CO_2.[5] In humans, the enzyme has been found to exist as two major isoenzymes, designated as HCAB and HCAC.[6,7] The high-resolution x-ray crystallographic data are available for both isoenzymes[8,9] and indicate considerable structural homology, with some subtle differences in the vicinity of the active site. Extensive reviews[10,11] are available which detail the physical, chemical, and enzymatic properties of the carbonic anhydrases.

Presented here are the Raman spectra of HCAB and apo-HCAB in H_2O and D_2O. Structural information deduced from the Raman data is found to provide a quite satisfactory agreement with that determined by x-ray diffraction.

Experimental Section

Carbonic anhydrase B was prepared by a modification[12] of the chloroform–ethanol method.[13] Samples of the native enzyme were prepared by dissolving lyophilized protein in either H_2O or D_2O containing 0.01 M Na_2SO_4 at a final pH (pD) of 6.9. The samples were equilibrated in solvent for several hours. Protein concentrations were about 15 mM. The enzyme was assayed for esterase activity as described by Armstrong et al.[15] using *p*-nitrophenyl acetate as substrate. The activity of the samples was the same both before and after spectra were taken.

The apoenzyme was prepared by dialysis against the chelating agent 1,10-phenanthroline as described by Lindskog and Nyman.[14] After