count for this new thermal isomerization. In the first step, a highly strained seven-membered heterocycle **2a** is formed via a [3,3]sigmatropic rearrangement of **1a.** This species may either give **3a** or return to **1a** by means of [3,3]sigmatropic shifts. Since the estimated heat of formation of **3a** (calculated by the method of Benson et al.[13]) is ~19 kcal mol^{-1} less than that of **1a,** the reaction proceeds in the expected direction, i.e., **1a** → **3a.** In contrast to the isomerization of *cis*-1-ethynyl-2-vinylcyclopropane,[3c] no dimers[14] were formed from the allenic intermediate **2a.** The above mechanism is supported by the analogous conversion of deuterated compound **1c** to **3c.** The structure of **3c** is confirmed by NMR: the spectrum reveals only one cyclopropane hydrogen at δ 1.85–1.50; moreover, the signal of the acetylenic hydrogen appears as a singlet.

Thermal rearrangement of **1b** to **3b** should also occur since the heat of formation of **3b** is estimated[13] to be less than ~9 kcal mol^{-1} that of **1b.** Nevertheless, only the formation of **4** is observed when **1b** is heated at 90 °C for 20 min. A pathway consistent with this fact would be a 1,3-hydrogen shift from the proposed intermediate **2b.** Since a thermal concerted 1,3-shift is forbidden by the Woodward–Hoffman rules,[15] we suggest that the hydrogen transfer occurs intramolecularly and is catalyzed by the nitrogen atom in **2b:** one of the two allylic hydrogens is near the nitrogen atom, because the six centered transition state leading to **2b** must generate a cis double bond. This hypothesis for the formation of the intermediate **2b** is further supported by the fact that **4** is the only product formed when *cis*-**3b**[16] is subjected to flow pyrolysis[10] at 350 °C.[17]

It may be asked why different pathways are observed when **1a** and **1b** are submitted to pyrolysis. This can be attributed to the higher basicity of the nitrogen atom over the oxygen atom.

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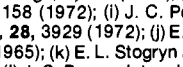
(6) CH_2=CHCH(NH-*t*-Bu)CH(OH)C≡CH, ^{1}H NMR (60 MHz, CCl_4, δ_{Me_4Si}) 1.15 (s, 9 H, *t*-Bu), 2.32 (d, 1 H, J = 2.32 Hz, HC≡), 2.9 (2 H, NH and OH) 3.26 (br t, 1 H, J = 7.5 Hz, HCN), 3.90 (2d, 1 H, J = 2.15 Hz, HCO) 5.03–5.38 (m, 2 H, CH_2=), 5.60–6.20 (m, 1 H, –CH=).

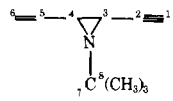
(7) (a) R. Appel and R. Kleinstuck, *Chem. Ber.,* **107,** 5 (1974); (b) J. C. Pommelet and J. Chuche, *Can. J. Chem.,* **54,** 1571 (1976).

(8) cis-**1a:** ^{1}H NMR (60 MHz, CCl_4, δ_{Me_4Si}) 2.32 (d, 1 H, J = 1.6 Hz, H_1),



cis-**1a**

3.26–3.50 (m, 2 H, H_3 and H_4), 5.16–6.00 (m, 3 H, H_5 and H_6). ^{13}C NMR (15.08 MHz, $CDCl_3$, δ_{Me_4Si}) 74.1 (d, C_1), 78.6 (d, C_2), 46.0 (d, C_3), 57.9 (d, C_4), 132.6 (d, C_5), 122.3 (t, C_6). MS (70 eV, *m/e*, rel intensity %) 94 (M^+, 5), 65 (100). cis-**1b:** ^{1}H NMR (60 MHz, CCl_4, δ_{Me_4Si}) 0.98 (s, 9 H, H_8), 1.88



cis-**1b**

(m, 1 H, H_5), 2.25 (m, 2 H, H_1 and H_3), 5.01–5.70 (m, 3 H, H_5 and H_6). ^{13}C NMR (15.08 MHz, $CDCl_3$, δ_{Me_4Si}) 69.0 (d, C_1) 82.0 (d, C_2), 26.9 (d, C_3), 39.9 (d, C_4), 136.2 (d, C_5), 118.0 (t, C_6), 54.1 (s, C_7), 26.3 (q, C_8). MS (70 eV, *m/e*, rel intensity %) 149 (M^+, 32), 93 (100).

(9) Spectral data for *trans*-**3a:** ^{1}H NMR (60 MHz, C_6H_6, δ_{Me_4Si}) 8.96 (d, 1 H, J = 3.8 Hz, H_5), 2.10–1.36 (m, 2 H, H_3 and H_4) 1.88 (d, 1 H, J = 1.6 Hz, H_1), 1.30–0.50 (m, 2 H, H_6). MS (70 eV, *m/e*, rel intensity %) 94 (M^+, 4), 65 (100).

(10) The products were dropped through a hot vertical Pyrex tube 80-cm in length; all pyrolyses carried out at ~15 Torr.

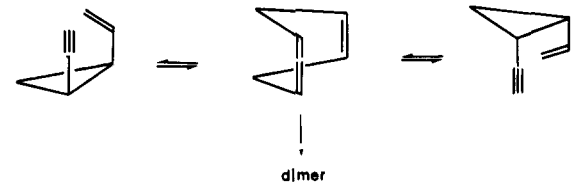
(11) L. A. Paquette, D. E. Kuhla, J. H. Barrett, and R. J. Haluska, *J. Org. Chem.,* **10,** 2866 (1969).

(12) The rates of disappearance of **1a** were determined in sealed tubes; 300 μL of a solution of **1a** in CCl_4 was placed in the tubes. The tubes were then frozen in dry ice–acetone, evacuated (0.1 Torr) and sealed off under nitrogen, while frozen. The contents of the tube were analyzed by NMR.

(13) S. W. Benson, "Thermochemical Kinetics", Wiley, New York, N.Y., 1968.

(14) It is likely that *cis*-1-ethynyl-2-vinylcyclopropane[3c] might undergo the degenerate rearrangement shown below.

Scheme III



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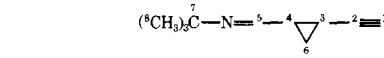
(16) **3b** was prepared in 45% yield by treatment of *cis*-**3a** with *tert*-butylamine and $CaCl_2$ in ether. ^{1}H NMR (60 MHz, $CDCl_3$, δ_{Me_4Si}) 7.30 (d, 1 H, J = 6.2



Hz, H_5), 2.25–1.60 (m, 2 H, H_3 and H_4) 1.95 (d, 1 H, H 1.8 Hz, H_1), 1.53–0.82 (m, 2 H, H_6), 1.20 (s, 9 H, H_8). ^{13}C NMR (15.08 MHz, $CDCl_3$, δ_{Me_4Si}) 67.4 (d, C_1), 82.9 (d, C_2), 6.9 (d, C_3), 22.7 (d, C_4), 158.6 (d, C_5), 14.3 (t, C_6), 57.1 (s, C_7), 29.8 (q, $_8$). MS (70 eV, *m/e*, rel intensity %) 149 (M^+, 28), 93 (100).

(17) Compound **3b** is stable under the milder conditions (90 °C) used for rearrangement of **1b.**

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The Crystal Structure of the Mushroom Toxin β-Amanitin[1]

Sir:

The deadly poisonous mushroom *Amanita phalloides* contains a number of cyclic peptides which can be classified as phallotoxins (heptapeptides), amatoxins (octapeptides), and antamanide, a decapeptide antagonist of the phallotoxins. The amatoxins cause death by destroying liver cells and damaging the secretory cells of the convoluted tubules in the kidney via inhibition of RNA polymerase II.[2,3] Although the chemical sequences of these cyclopeptides have been determined, only antamanide has been subjected to a three-dimensional structure analysis.[4]

We wish to report the x-ray crystallographic structure determination of the amatoxin β-amanitin, isolated and purified from American *Amanita phalloides.*[5] β-Amanitin (**1**), $C_{39}H_{53}SO_{15}N_9$, has the chemical sequence cyclo (L-α-aspartyl-4-hydroxy-L-prolyl-4,5-dihydroxy-L-isoleucyl-6-hydroxy-2-mercapto-L-tryptophyl-glycyl-L-isoleucyl-glycyl-L-cysteinyl) cyclo(4 → 8)-*S*-oxide. The octapeptide ring is bridged through the sulfur atom of the sulfoxide form of cysteine to the 2 position of the indole ring. The resulting bicyclic structure contains two 18-membered rings.

Crystals were grown by slow evaporation from a 95% eth-

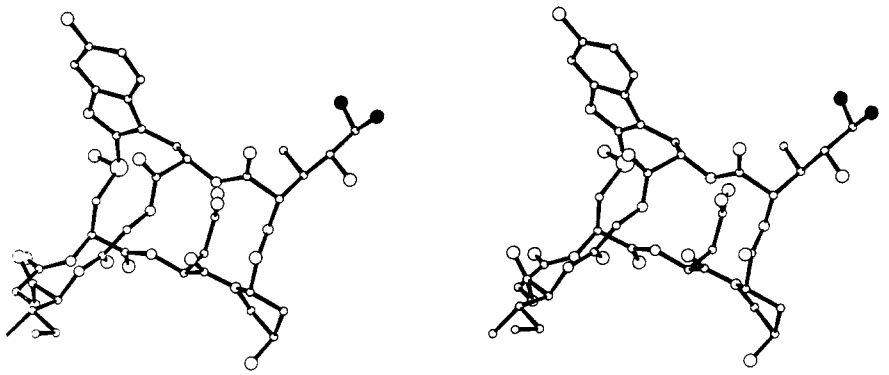
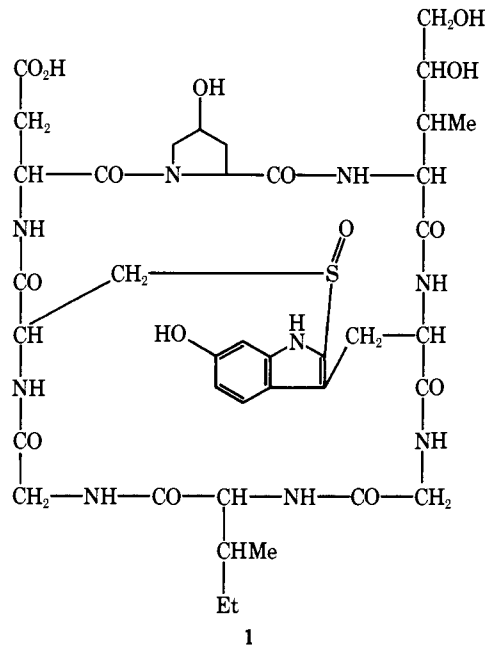


Figure 1. A stereoscopic view of β-amanitin. The dark atoms represent disordered positions of the terminal hydroxy group. At the right there is a single turn which is very nearly α-helical.



1

anol solution and have the symmetry of the orthorhombic space group $P2_12_12_1$. The unit cell has dimensions a = 14.004 (3), b = 14.943 (3), and c = 30.794 (7) Å and contains four β-amanitin molecules. Crystals for data collection had to be sealed in glass capillaries with a small amount of mother liquor to prevent deterioration. The integrated intensities of 4104 independent reflections ($2\theta_{max}$ = 105°) were measured with graphite monochromated Cu Kα radiation on an automated four-circle diffractometer. Reflection intensities were corrected for background, polarization, and Lorentz effects. Those 3395 reflections (83%) which had values of $F_o \geq 3\sigma(F_o)$ were considered to be observed.

An initial structural model consisting of the peptide backbone and half of the side chains was obtained using the weighted multiple solution tangent formula approach of direct methods.[6] The remaining side-chain atoms were located from an F_o map. Several molecules of ethanol and water exhibiting considerable thermal motion and disorder were added to the model by means of successive least-squares refinement cycles and ΔF syntheses. Block-diagonal matrix least-squares refinement utilizing anisotropic temperature factors and a fractional weighting scheme[7] has reduced the standard crystallographic R to 11% at present; further refinement is in progress. Hydrogen atoms have not been included in the model. Bond distances and angles are near expected values.

All amino acid residues have the L configuration and the C^β atom of the isoleucine is S as expected. The crystallographic results confirm the latest chemical structural work assigning the R configuration to the C^β and C^γ atoms of the dihydroxyisoleucine[8] and to the sulfur of the cysteine.[9,10] In addition, the configuration at the C^γ atom of the hydroxyproline is established as R. Figure 1 is a computer generated stereoscopic view[11] of the molecule. The associated solvent molecules and hydrogen atoms are omitted for clarity. The terminal hydroxy group of the dihydroxyisoleucine is shown to have two positions because of disorder. Three intramolecular hydrogen bonds are present: Asp_1 O^δ to Trp_4 NH, Asp_1 carbonyl O to Gly_5 NH, and Gly_5 carbonyl O to Cys_8 NH. All peptide bonds are in the trans conformation.

Full details of the purification of β-amanitin and determination of its crystal structure will be published at a later date.

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