Structure and Conformation of Roseotoxin B

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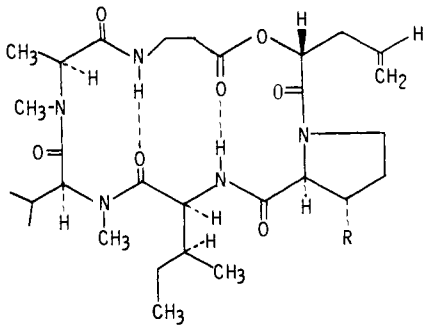
Abstract: The structure and conformation of the toxic metabolite roseotoxin B from *Trichothecium roseum* are presented. X-ray diffraction data on two different crystal forms and NMR data are given in support of the structural assignment. Crystals from benzene had cell constants of a = 10.110 (3) Å, b = 10.590 (2) Å, c = 18.563 (2) Å, and β = 105.84 (2)° in space group $P2_1$ and the X-ray data refined to an unweighted residual (R) of 0.089. Crystals from ether/hexane were also of space group $P2_1$ and were examined at 138 K and at room temperature. At 138 K, the cell constants were a = 9.516 (3) Å, b = 10.734 (5) Å, c = 16.495 (6) Å, and β = 101.72 (4)° and the data refined to an R of 0.039, while at room temperature, the cell constants were a = 9.7759 (10) Å, b = 10.7853 (12) Å, c = 16.692 (2) Å, and β = 102.88 (10)° and the final R was 0.092. The current work corrects the amino acid and hydroxy acid sequence reported previously. Conformational differences of the three structures are noted. In addition, the solid-state and solution conformations are compared to the conformation of the related destruxins proposed from chemical and physical methods. Roseotoxin B has an oral LD_{50} in day-old chickens of 12.5 mg/kg.

During the course of the investigation of toxic metabolites produced by fungi growing on acids, foodstuffs, we (Springer, Cole, Dorner, Dox, and Richards) became interested in the nature of a particular toxic isolate of *Trichothecium roseum* found on improperly stored peanuts. *T. roseum* has been frequently isolated from stored foodstuffs and is capable of producing a wide variety of metabolites including trichothecane mycotoxins such as trichothecin and trichothecolone as well as rosolactone, ergosterol, and rosenonolactone. Systematic fractionation of culture isolates from our toxigenic isolates of *T. roseum* led to the isolation of rosenonolactone, trichothecolone, and the quite toxic roseotoxin B (**1**). Independently, the group at Oklahoma obtained a specimen of roseotoxin B from Dr. G. W. Engstrom and pursued a structure determination as part of a continuing study of fungal peptide products, cyclic peptides and cyclic depsipeptides.



Roseotoxin B (1) R = CH_3

Destruxin A (2) R = H

Previous work[2] had determined that roseotoxin B (**1**) was composed of the five amino acids, i.e., *trans*-3-methylproline, isoleucine, *N*-methylvaline, β-alanine, and *N*-methylalanine as well as the hydroxy acid 2-hydroxy-4-pentenoic acid. The absolute configuration of the isoleucine was shown to be *S* by oxidation with L-amino acid oxidase. In addition, a cyclic sequence was proposed on the basis of degradation experiments;[3] however, the order of the β-alanine and the *N*-methylalanine in the proposed sequence was questionable, especially when compared to the related destruxins.[4] Also, the configuration of the stereochemical centers other than L-isoleucine were not identified. Furthermore, since NMR experiments indicated that one conformation predominated in solution, the solid-state conformation was of particular interest. We wish to describe our recent work on the structure and conformation of roseotoxin B (**1**), a toxic, fungal cyclodepsipeptide.

Experimental Section

High-resolution electron-impact mass spectral analysis was made with an A.E.I. MS-902 double focusing instrument. The ion source temperature was kept at 200 °C and high-resolution measurements were made by peak matching with perfluorokerosene as an internal standard. Ultraviolet spectra were measured in methanol with a Beckman Model DB-6 recording spectrophotometer. Infrared spectra in Nujol were taken with a Perkin-Elmer Model 257 IR spectrophotometer equipped with a 4X beam condenser. ^1H NMR experiments were run using a Bruker WM-250 instrument with Me_4Si as internal standard. Melting points were determined on a Kofler hot point apparatus and are uncorrected. Thin-layer chromatography was performed on silica gel G-HR with toluene–ethyl acetate–formic acid (5: 4: 1 v/v/v) and visualizing with *p*-(dimethylamino)benzaldehyde followed by 50% ethanolic sulfuric acid and heat.

T. roseum was isolated from moldy peanuts by surface sterilization of peanuts with 1% NaOCl for 2 min followed by incubation on Littman Oxgall agar for 5 days at 25 °C. A pure isolate was taken, grown an additional 5 days in still culture on potato dextrose agar at 25 °C, and then maintained at 5 °C. Toxigenicity of the fungus was determined by the method of Kirksey and Cole[5] and Cole.[6]

Forty Fernbach flasks (2.8 L), each containing approximately 100 g of shredded wheat supplemented with 200 mL of Difco mycological broth (pH 4.8) plus YES medium,[7] were used to mass culture the fungus. After a 2-week incubation the fungal growth was homogenized, extracted with hot chloroform, and concentrated. Purification of the fungal metabolites was achieved through the use of column chromatography and crystallization with each step monitored by bioassay and thin-layer chromatography. Day-old chickens were used for bioassay, and samples

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(1 mg/chicken) were administered via crop intubation with corn oil serving as the inert carrier.

Three compounds were isolated and characterized during the course of this investigation. The first was found to be nontoxic and identified to be rosenonolactone (mp 220–222 °C) by comparison of the UV, IR, ^{1}H NMR, ^{13}C NMR, and mass spectra with those reported previously.[8,9] The second metabolite (mp 177–178 °C) was mildly toxic (350 mg/kg) and identified as the sesquiterpene trichothecolone[10] by comparison with an authentic compound. Finally, the highly toxic (12 mg/kg) metabolite **1** was isolated and purified. The toxin was soluble in chloroform, carbon tetrachloride, ethyl ether, ethyl acetate, benzene, and ethyl alcohol while sparingly soluble in hot water and insoluble in hydrocarbons. The thin-layer chromatography of the compound revealed one spot at R_f 0.48. The infrared spectra indicated amide and ester functionality (ν_{max}^{KBr} 1730, 1665, 1630, 1510–1540, and 1178 cm^{-1}). High-resolution mass spectroscopy gave a molecular ion at m/e 591.3621 (calculated for $C_{30}H_{49}O_7N_5$ 591.3632). Other prominent peaks were fragment ions at m/e 576 ($M^+ - CH_3$), 534 ($M^+ - C_4H_9$), and 506 ($M^+ - C_5H_{11}N$). These data, in addition to ^{1}H NMR data, were consistent with a previously described fungal metabolite named roseotoxin B (**1**).[3]

Crystals of **1** formed from benzene were large, clear, and chunky. Preliminary X-ray diffraction experiments indicated a symmetry of $P2_1$ with cell lengths of a = 10.110 (3) Å, b = 10.590 (2), c = 18.563 (2), and β = 105.84 (2)°. The X-ray diffraction measurements were made with a Syntex $P2_1$automatic 4-circle diffractometer using Cu K$\bar{\alpha}$ (λ = 1.5418 Å) radiation. Of the 2747 reflections measured with $2\theta \lesssim 114°$, 2323 were observed ($I \gtrsim 3\sigma I$). The structure was solved through judicious application of a multisolution tangent formula approach to deriving the initial phases.[11] Structural refinement was accomplished through the use of Fourier refinements and least-squares techniques. A disordered molecule of benzene was found in the crystal lattice and was modeled with 12 atoms with occupancy factors of 0.5. All temperature parameters except those of the benzene were refined anisotropically. The function $\sum w(|F_o| - |F_c|)^2$ with $w = 1/(\sigma F_o)^2$ was minimized to an unweighted R factor of 0.089[12] and a weighted R factor of 0.098. The standard deviation of an observation of unit weight was 5.58 while the data/parameter ratio was 5.45. Hydrogens were not included in the refinement.

Crystals of **1** formed from diethyl ether/hexane were large clear rods and were also found to be of space group $P2_1$, but with a = 9.516 (3) Å, b = 10.734 (5) Å, c = 16.495 (6) Å, and β = 101.72 (4)° at 138 K and a = 9.7759 (10) Å, b = 10.7853 (12) Å, c = 16.692 (2) Å, and β = 102.88 (10)° at room temperature. Data were taken on an Enraf-Nonius CAD 4 diffractometer fitted with alow-temperature apparatus, using Cu K$\bar{\alpha}$ radiation. At low temperature, 3563 reflections were measured with $2\theta \lesssim 150°$, of which 3537 were considered observed ($I \gtrsim 2\sigma I$), but at room temperature the data were considerably weaker, and of 3714 reflections with $2\theta \lesssim 150°$, 2430 were considered observed ($I \gtrsim 2\sigma I$). The structure was solved by use of direct methods[11] on the low-temperature data. Following initial refinement of the model, it proved possible to locate all hydrogen atoms in a difference Fourier map. Block-diagonal least-squares refinement of the model with anisotropic thermal parameters for the non-hydrogen atoms and isotropic thermal parameters for the hydrogen atoms gave a final unweighted R factor of 0.039.[13] The standard deviation of an observation of unit weight was 1.60 and the data/parameter ratio was 5.55. The final atomic coordinates, excluding the hydrogen atoms, were then used as a starting model for refinement with the room-temperature data. Anisotropic refinement of the non-hydrogen atoms by blocked-full-matrix methods gave a final R factor of 0.092 and weighted R of 0.106.[14] The standard deviation of an observation of unit weight was 3.41 and the data/parameter ratio was 6.43. The three structures are designated RB-RT (crystals from benzene, room temperature), REH-LT (crystals from ether/hexane, 138 K), and REH-RT (crystals from ether/hexane, room temperature). Final atomic coordinates for RB-RT and REH-LT are given in Tables I and II. Tables of atomic coordinates for REH-RT, distances and angles for RB-RT and REH-RT, hydrogen atom parameters, and structure factors may be found in the supplementary material. In both room temperature studies, some distances and angles, especially for the side chains, show deviations from ideality.

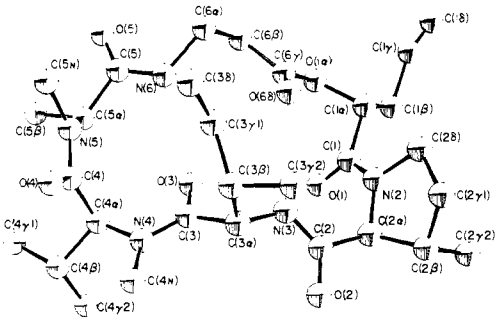

Figure 1. Perspective drawing of roseotoxin B as found in RB-RT. Atomic spheres are drawn on an arbitrary scale.

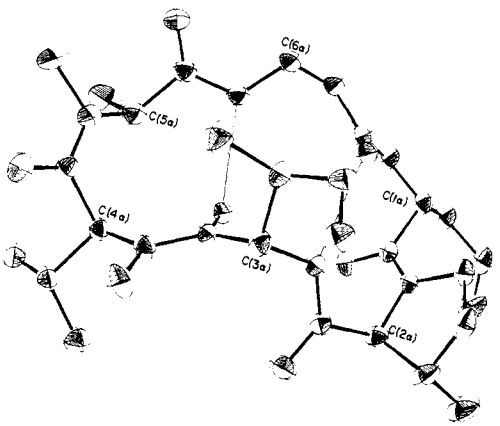

Figure 2. Perspective drawing of roseotoxin B as found in REH-LT. Thermal ellipsoids are drawn at the 50% probability level.

A third crystal form of **1** was obtained from diethyl ether/petroleum ether mixtures. These crystals displayed trigonal diffraction symmetry with approximate cell dimensions $a = b$ = 25.1 Å and c = 15.7 Å. The cell volume (V_c = 8600 Å^3) suggested three independent molecules in the asymmetric unit. The quality of the crystals was not such that it would be possible to collect good high-angle diffraction data; therefore, a structure solution for this crystal form was not pursued.

The absolute configuration of roseotoxin B (**1**) as assigned from the experimentally determined stereochemistry of the isoleucine was checked with the Bijvoet method for the anomalous dispersion of the Cu radiation by the seven oxygen atoms in the molecule, using the REH-LT crystal and structure. The general procedure for the selection of the proper Friedel pairs and subsequent measurements has been detailed elsewhere.[15] Briefly, this involved the repetitive measurement of the intensities for the hkl and $h\bar{k}l$ of each reflection shown to have a high sensitivity factor [SF = $\{F^2(+) - F^2(-)\}/\sigma(I)$]. The intensities of 18 such reflections were measured 15 times at each position. The observed and calculated values for these parameters are given in Table XII of the supplementary material. Of the 18 reflections, 15 agree with the absolute configuration reported.

Results and Discussion

The structure and conformations for roseotoxin B (**1**) as found in RB-RT and REH-LT are shown in Figures 1 and 2, respectively. The correct sequence of hydroxy and amino acids for roseotoxin B (**1**) *cyclo*(2(*R*)-hydroxy-4-pentenoyl-*trans*-3-methyl-L-prolyl-L-isoleucyl-*N*-methyl-L-valyl-*N*-methyl-L-alanyl-β-alanyl). This reverses the previous assignment of the order of *N*-methylalanine and β-alanine, which makes **1** a member of the destruxins. Roseotoxin B (**1**) is identical with destruxin A (**2**)[4] except that the proline in **2** is replaced by *trans*-3-methylproline in **1**. In addition, the absolute configuration of all the stereochemical centers is now established. Since the isoleucine is known to have the normal *S* (L) configuration, the configuration of the remainder of the amino acids is also *S* (L) while the hydroxy acid is *R* (D). As a check, the absolute configuration was determined by use of the Bijvoet method for the anomalous dispersion

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Table I. RB-RT Positional Parameters (×10⁴) and Equivalent Isotropic Temperature Factor (×10³)[a]

	x	y	z	U_{eq}, Å² [b]
O(1α)	6329 (5)	3796 (6)	4757 (3)	66 (4)
C(1α)	5418 (9)	3856 (11)	5281 (4)	67 (6)
C(1)	4177 (9)	2984 (9)	4939 (5)	64 (6)
O(1)	4300 (6)	1936 (6)	4702 (3)	70 (4)
C(1β)	6263 (10)	3263 (12)	6003 (5)	88 (7)
C(1γ)	7528 (15)	4194 (23)	6364 (7)	159 (13)
C(1δ)	8167 (21)	4666 (20)	6751 (11)	208 (17)
N(2)	2934 (7)	3485 (7)	4942 (3)	56 (4)
C(2α)	1704 (8)	2725 (9)	4732 (4)	57 (5)
C(2)	968 (8)	2802 (9)	3871 (4)	57 (5)
O(2)	−214 (6)	2351 (8)	3679 (3)	79 (4)
C(2β)	787 (9)	3315 (10)	5185 (4)	79 (5)
C(2γ1)	1128 (9)	4752 (12)	5164 (5)	86 (6)
C(2γ2)	1286 (11)	2769 (13)	6025 (5)	101 (7)
C(2δ)	2715 (10)	4768 (9)	5247 (5)	78 (6)
N(3)	1606 (6)	3324 (7)	3425 (3)	55 (4)
C(3α)	922 (8)	3387 (9)	2632 (4)	49 (4)
C(3)	1790 (7)	2676 (8)	2201 (4)	51 (4)
O(3)	3056 (5)	2589 (6)	2481 (3)	55 (3)
C(3β)	671 (9)	4780 (10)	2303 (5)	74 (6)
C(3γ1)	2043 (10)	5437 (11)	2238 (6)	101 (7)
C(3γ2)	−4 (9)	5566 (10)	2835 (5)	97 (7)
C(3δ)	1812 (15)	6620 (13)	1752 (8)	139 (11)
N(4)	1196 (6)	2234 (7)	1513 (3)	45 (3)
C(4N)	−304 (8)	2326 (11)	1142 (4)	62 (5)
C(4α)	2151 (8)	1788 (9)	1091 (4)	53 (4)
C(4)	2447 (8)	2932 (9)	652 (4)	58 (5)
O(4)	1523 (6)	3515 (8)	217 (3)	94 (4)
C(4β)	1481 (9)	700 (10)	508 (5)	72 (6)
C(4γ1)	2506 (10)	377 (13)	43 (6)	120 (8)
C(4γ2)	1220 (11)	−402 (11)	975 (7)	92 (7)
N(5)	3746 (7)	3416 (7)	797 (3)	62 (4)
C(5N)	3944 (11)	4632 (11)	404 (5)	88 (6)
C(5α)	5015 (8)	2765 (10)	1223 (5)	62 (5)
C(5)	5958 (8)	3691 (10)	1787 (5)	69 (5)
O(5)	7059 (6)	3973 (9)	1700 (4)	111 (5)
C(5β)	5766 (9)	2170 (12)	677 (5)	96 (6)
N(6)	5469 (7)	4058 (0)	2332 (4)	76 (5)
C(6α)	6180 (10)	5089 (12)	2904 (5)	86 (7)
C(6β)	6858 (9)	4437 (12)	3639 (5)	80 (6)
C(6γ)	5794 (8)	4312 (9)	4095 (5)	64 (5)
O(6δ)	4610 (6)	4633 (7)	3872 (3)	70 (4)
Coordinates of Disordered Benzene (×10³)-Isotropic U				
C1	236 (2)	501 (2)	770 (1)	90 (7)
C2	215 (2)	421 (2)	799 (1)	84 (7)
C3	353 (2)	328 (2)	825 (1)	104 (7)
C4	433 (2)	358 (2)	809 (1)	78 (7)
C5	455 (2)	455 (3)	768 (1)	137 (9)
C6	343 (3)	539 (3)	738 (1)	137 (10)
C7	266 (2)	548 (2)	739 (1)	90 (7)
C8	181 (3)	484 (2)	778 (2)	145 (>10)
C9	271 (2)	361 (3)	825 (1)	113 (8)
C10	467 (4)	297 (5)	816 (2)	291 (>10)
C11	481 (2)	415 (3)	785 (1)	111 (9)
C12	411 (2)	520 (3)	743 (1)	100 (8)

[a] Esd's are in parentheses. [b] $U_{eq} = (U_{11} \times U_{22} \times U_{33})^{1/3}$.

Table II. REH-LT Positional Parameters (×10⁴) and Equivalent Isotropic Temperature Factor (×10⁴) for non-H Atoms[a]

	x	y	z	U_{eq} [b]
O(1α)	3429 (2)	129 (2)	5337 (1)	347 (7)
C(1α)	4349 (2)	367 (2)	4745 (1)	286 (8)
C(1)	5706 (2)	−435 (2)	4979 (1)	236 (7)
O(1)	5621 (2)	−1541 (2)	5148 (1)	328 (6)
C(1β)	3475 (3)	−37 (3)	3904 (1)	380 (10)
C(1γ)	4281 (3)	217 (3)	3229 (1)	404 (10)
C(1δ)	4872 (3)	−656 (4)	2851 (2)	522 (14)
N(2)	6971 (2)	132 (2)	+975 (1)	218 (6)
C(2α)	8310 (2)	−575 (2)	5161 (1)	251 (8)
C(2)	8878 (2)	−777 (2)	6092 (1)	226 (7)
O(2)	9931 (2)	−1441 (2)	6337 (1)	363 (7)
C(2β)	9339 (2)	264 (3)	4801 (1)	351 (10)
C(2γ1)	8840 (3)	1569 (3)	4971 (1)	366 (10)
C(2γ2)	9212 (3)	32 (4)	3876 (1)	522 (14)
C(2δ)	7201 (2)	1456 (2)	4794 (1)	294 (8)
N(3)	8218 (2)	−131 (2)	6596 (1)	232 (6)
C(3α)	8649 (2)	−229 (2)	7492 (1)	272 (8)
C(3)	8042 (2)	−1417 (2)	7785 (1)	229 (7)
O(3)	6808 (2)	−1740 (2)	7447 (1)	265 (6)
C(3β)	7995 (2)	929 (2)	7866 (1)	330 (9)
C(3γ1)	8536 (3)	2142 (2)	7533 (2)	367 (9)
C(3γ2)	8288 (3)	928 (3)	8814 (1)	393 (10)
C(3δ)	10133 (3)	2207 (3)	7627 (2)	395 (11)
N(4)	8834 (2)	−2047 (2)	8424 (1)	261 (7)
C(4N)	10332 (2)	−1704 (3)	8796 (2)	387 (10)
C(4α)	8139 (2)	−3080 (2)	8780 (1)	250 (7)
C(4)	7710 (2)	−2576 (2)	9567 (1)	287 (8)
O(4)	8652 (2)	−2453 (2)	10192 (1)	416 (7)
C(4β)	9074 (2)	−4248 (2)	8977 (1)	290 (8)
C(4γ1)	8164 (3)	−5298 (2)	9226 (1)	367 (9)
C(4γ2)	9708 (3)	−4635 (2)	8238 (1)	345 (9)
N(5)	6334 (2)	−2236 (2)	9561 (1)	328 (8)
C(5N)	6052 (4)	−1847 (3)	10366 (2)	524 (14)
C(5α)	5090 (2)	−2405 (2)	8868 (1)	316 (9)
C(5)	4273 (2)	−1169 (2)	8647 (1)	302 (9)
O(5)	3534 (2)	−718 (2)	9096 (1)	441 (8)
C(5β)	4082 (3)	−3404 (3)	9062 (2)	541 (14)
N(6)	4361 (2)	−690 (2)	7905 (1)	323 (8)
C(6α)	3590 (3)	443 (3)	7589 (1)	366 (10)
C(6β)	2920 (2)	327 (3)	6667 (1)	405 (11)
C(6γ)	3992 (2)	484 (2)	6116 (1)	299 (8)
O(6δ)	5192 (2)	899 (2)	6333 (1)	318 (6)

[a] Esd's are in parentheses. [b] $U_{eq} = {}^1/_6\pi^2 \Sigma_i \Sigma_j \beta_{ij} \bar{a}_i \bar{a}_j$.

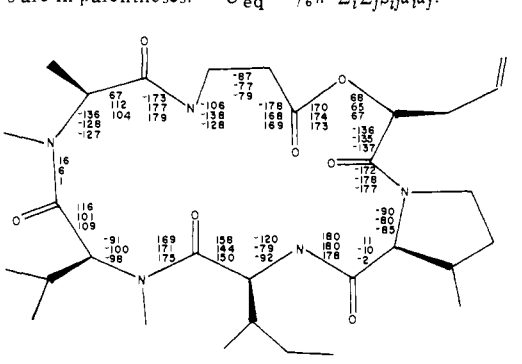

Figure 3. Dihedral angles for the backbone of roseotoxin B as observed in RB-RT (top), REH-LT (middle), and REH-RT (bottom). Estimated standard deviations are 0.6–0.9° for the room-temperature structures (RB-RT and REH-RT) and 0.2–0.4° for the low-temperature structure (REH-LT).

of the Cu radiation by all oxygen atoms in the molecule[15] with the REH-LT data and was found to agree with the proposed stereochemistry.

All of the amide and ester bonds are trans except for the cis amide between the *N*-methylvaline and *N*-methylalanine. A cis amide is not unusual because cis conformations have been observed in peptides with secondary amides involving proline and *N*-methyl amino acids. In fact, a cis amide was speculated to exist in a related destruxin from NMR experiments[16] and by analogy with the crystal structure of ilamycin B which also has *N*-methyl amide bonds.[17]

Two significant intramolecular NH···O hydrogen bonding contacts of the type 4→1 exist in both the RB-RT and REH-LT crystal structures (and in the REH-RT structure, but the much more reliable REH-LT distances are quoted herein). One involves N(6) to O(3) of lengths 2.99 (RB-RT) and 2.824 Å (REH-LT), and the other involves N(3) to O(6δ) of lengths 3.22 (RB-RT) and 3.033 Å (REH-LT). These two hydrogen bonds make two 10-membered rings inside the covalent 19-membered ring. As a consequence, the two ends of the molecule are constrained to β-turns, an observation consistent with the sharp resonances in

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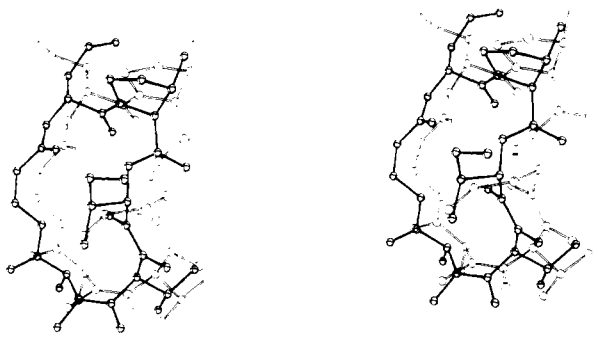

Figure 4. Side-by-side stereoview of REH-LT and RB-RT. RB-RT is to the right, drawn with hollow bonds and plain ellipsoids.

the solution NMR. An alternative interpretation of the function of hydrogen bonds in the β-turn conformation has recently been suggested by Snyder.[18] Using molecular mechanics methodology, Snyder has shown that such bonds are more accurately described as the result of the β-turn geometry than as the cause for such a geometry. Conformational angles descriptive of the two turns of the molecule are given in Figure 3. By use of notation provided by Karle,[19] the ring containing N(6) and O(3) would be classified a 4→1 cis interaction by virtue of the dihedral angles. This interaction has a precedent in ilamycin B,[17] which has two similar sets of dihedral angles. The second turn containing N(3) and O(6δ) would be called 4→1 trans, type II′, which has a very close precendent in valinomycin.[20]

The macrocyclic backbones of cyclic peptide and cyclic depsipeptide molecules are quite flexible structures. The conformations of roseotoxin B (**1**) in the structures RB-RT and REH-LT may be compared in the side-by-side stereoview of Figure 4 and from the dihedral angles given in Figure 3. The most dramatic differences in the molecular conformations are in the pentenoic acid and isoleucine side chains, but there are differences in the 19-membered ring conformations as well. These differences are greater than 20° in the dihedral angles about the C(2α)–C(2), N(3)–C(3α), C(5α)–C(5), and N(6)–C(6α) bonds. There are no intermolecular hydrogen bonds in the structures and the molecular packing in the crystals is directed by van der Waals contacts. The RB-RT structure has a disordered benzene cocrystallized with the mycotoxin, and it is to be expected that differences in the resultant packing forces would be reflected in the molecular conformation of the mycotoxin. The backbone dihedral angles in the region of the isolecine residue are quite different between the two structures, but those adjacent to the pentenoic acid vary only slightly, indicating that the effects of varying packing forces are distributed about the molecule. The β-alanine lends an extra degree of freedom to the backbone and the differences in the dihedral angles about the N(6)–C(6α) and C(6α)–C(6β) bonds tend to cancel each other.

Another potential source of conformational differences between the RB-RT and REH-LT structures is the different temperatures at which the studies were done. For this reason we undertook a room-temperature study of the REH-LT crystal form. The conformational angles found for this structure, REH-RT, are also given in Figure 3. Though it should be stressed that derived results from the room-temperature studies are not as reliable as those of the low-temperature study, it is interesting to note that the conformational angles of REH-RT, though closer to those of REH-LT, are generally between the values found in RB-RT and REH-LT.

Two important conclusions can therefore be made. In the first place, it does not appear to be possible in roseotoxin B to make a clear distinction between the influence of temperature and the van der Waals' forces on the conformation of the molecule. Secondly, the overall shape of the molecule, with two turns forming 10-membered rings by 4→1 hydrogen bonds, is conserved in both crystal forms despite significant differences in the conformational angles about the 19-membered backbone. Thus, it appears that the intramolecular hydrogen bonds and N-methylation restrict, but do not eliminate, the conformational freedom of the molecule in accordance with findings of solution NMR studies. This is also supported by NMR studies in the destruxin series where one member lacking N-methylation is much more flexible in solution and does not appear to form stable transannular H bonds.[16]

The bond distances and angles for REH-LT are given in Figure 5 and Table III. As is usual in peptide structures, there is a considerable range in the values of formally equivalent bond lengths and angles of the peptide units, amounting to several esds in some cases, though the average values are in general agreement with the reported mean values for several peptide structures.[21] The largest deviations in bond distances and angles from the reported mean values, i.e., N(4)–C(4α) 1.473 Å, N(5)–C(5α) 1.480 Å, and the bond angles around N(4), N(5), C(4), and C(5),

Table III. Bond Angles for REH-LT[a]

C(6γ)-O(1α)-C(1α)	114.5	C(3α)-C(3β)-C(3γ2)	113.3
O(1α)-C(1α)-C(1)	108.7	C(3γ1)-C(3β)-C(3γ2)	111.1
O(1α)-C(1α)-C(1β)	105.5	C(3β)-C(3γ1)-C(3δ)	114.1
C(1)-C(1α)-C(1β)	110.2	C(3)-N(4)-C(4α)	117.6
C(1α)-C(1)-O(1)	120.6	C(3)-N(4)-C(4N)	122.6
C(1α)-C(1)-N(2)	116.8	C(4α)-N(4)-C(4N)	119.7
O(1)-C(1)-N(2)	122.6	N(4)-C(4α)-C(4)	106.8
C(1α)-C(1β)-C(1γ)	110.8	N(4)-C(4α)-C(4β)	114.3
C(1β)-C(1γ)-C(1δ)	123.7	C(4)-C(4α)-C(4β)	110.5
C(1)-N(2)-C(2α)	120.4	C(4α)-C(4)-O(4)	118.3
C(1)-N(2)-C(2δ)	127.1	C(4α)-C(4)-N(5)	120.6
C(2α)-N(2)-C(2δ)	112.5	O(4)-C(4)-N(5)	121.1
N(2)-C(2α)-C(2)	113.5	C(4α)-C(4β)-C(4γ1)	108.9
N(2)-C(2α)-C(2β)	102.2	C(4α)-C(4β)-C(4γ2)	110.0
C(2)-C(2α)-C(2β)	110.3	C(4γ1)-C(4β)-C(4γ2)	110.6
C(2α)-C(2)-O(2)	120.6	C(4)-N(5)-C(5α)	126.3
C(2α)-C(2)-N(3)	115.8	C(4)-N(5)-C(5N)	115.0
O(2)-C(2)-N(3)	123.5	C(5α)-N(5)-C(5N)	118.0
C(2α)-C(2β)-C(2γ1)	102.7	N(5)-C(5α)-C(5)	111.2
C(2α)-C(2β)-C(2γ2)	111.4	N(5)-C(5α)-C(5β)	111.4
C(2γ1)-C(2β)-C(2γ2)	111.7	C(5)-C(5α)-C(5β)	110.3
C(2β)-C(2γ1)-C(2δ)	103.6	C(5α)-C(5)-O(5)	121.5
N(2)-C(2δ)-C(2γ1)	103.0	C(5α)-C(5)-N(6)	114.9
C(2)-N(3)-C(3α)	121.6	O(5)-C(5)-N(6)	123.4
N(3)-C(3α)-C(3)	109.7	C(5)-N(6)-C(6α)	121.5
N(3)-C(3α)-C(3β)	106.8	N(6)-C(6α)-C(6β)	111.4
C(3)-C(3α)-C(3β)	109.4	C(6α)-C(6β)-C(6γ)	113.2
C(3α)-C(3)-O(3)	118.5	C(6β)-C(6γ)-O(6δ)	125.2
C(3α)-C(3)-N(4)	118.8	C(6β)-C(6γ)-O(1α)	111.0
O(3)-C(3)-N(4)	122.6	O(6δ)-C(6γ)-O(1α)	123.8
C(3α)-C(3β)-C(3γ1)	109.9		

[a] Esd's are 0.2–0.3°.

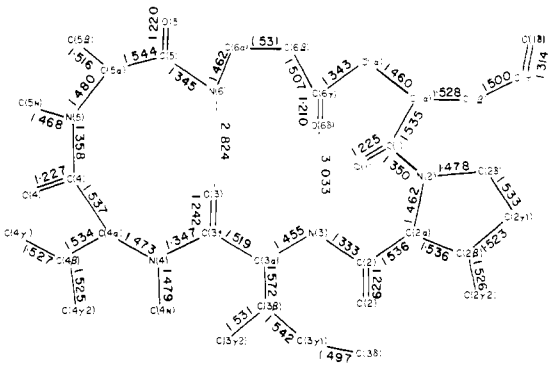

Figure 5. Bond distances for REH-LT. Estimated standard deviations are 0.002–0.004 Å.

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Table IV. Conformational Parameters for the Peptide Units (REH-LT)[a]

	Pentenoyl-MePro	MePro-Ile	Ile-MeVal	MeVal-MeAla	MeAla-βAla
$\omega_1 = C\alpha_i - C'_i - N_{i+1} - C\alpha_{i+1}$	−177.8 (1)	−179.8 (2)	170.7 (2)	6.3 (3)	177.0 (2)
$\omega_2 = O_i - C'_i - N_{i+1} - H^b$	−179.1 (2)	−165.7 (22)	176.5 (2)	−6.1 (3)	164.2 (21)
$\omega_3 = O_i - C'_i - N_{i+1} - C\alpha_{i+1}$	1.1 (2)	−4.1 (3)	−6.1 (3)	−176.1 (2)	1.6 (4)
$\omega_4 = C\alpha_i - C'_i - N_{i+1} - H^b$	2.0 (2)	18.6 (23)	−6.6 (3)	176.3 (2)	−20.4 (21)
$\tau' = \omega_i + \omega_2, \lvert\omega_1 - \omega_2\rvert < \pi$	3.1 (3)	14.5 (24)	−12.8 (4)	0.2 (6)	−18.8 (23)
$\chi_C = \omega_1 - \omega_3 + \pi$ Mod 2π	1.1 (3)	4.3 (5)	−3.2 (5)	2.4 (5)	−4.6 (6)
$\chi_N = \omega_2 - \omega_3 + \pi$	−0.2 (4)	18.1 (25)	2.6 (5)	−10.0 (5)	−17.6 (25)

[a] In degrees. Standard deviations for last digits in parentheses. [b] Or C for methylproline, *N*-methylvaline and *N*-methylalanine.

Table V. ^{1}H 250-MHz Chemical Shifts and Coupling Constants Assignments

amino acid	ppm downfield from Me_4Si (coupling information)
N-methylalanine	
αCH	5.14 (J = 7.0, q)
CH_3	1.31 (J = 7.0, d)
NCH_3	2.72 (s)
N-methylvaline	
αCH	4.95 (J = 11.0, d)
βCH	2.31 (m)
CH_3X2	0.92 (J = 6.7, d), 0.84 (J = 7.0. d)
NCH_3	3.22 (s)
2-hydroxy-4-pentenoic acid	
αCH	4.84 (J = 4.1, 7.0, dd)
βCH_2	2.65, 2.74 (m)
γ=CH	5.78 (J = 7.0, 9.9, 17.2, m)
$\delta=CH_2$	5.22 (J = 1.5, 17.2, dd), 5.16 (J = 1.5, 9.9, dd)
β-alanine	
NH	8.24 (J = 8.1, d)
αCH_2	1.88 (m), 1.42 (m)
βCH_2	4.04 (J = 4.8, 7.0, 9.1, 13.2, m), 3.06 (m)
isoleucine	
NH	7.04 (J = 9.2, d)
αCH	4.84 (J = 9.2, 7.0, dd)
βCH	2.09 (m)
βCH_3	0.88 (J = 6.3, d)
γCH_2	2.65 (m)
δCH_3	0.85 (J = 7.3, t)
3-methylproline	
αCH	4.26 (J = 2.1, d)
βCH	2.74 (m)
βCH_3	1.08 (J = 7.3, d)
γCH_2	1.67 (m), 2.09 (m)
δCH_2	3.59 (m), 3.82 (m)

seem to be associated with either the *N*-methyl groups or the cis peptide linkage. The relatively long C(3)–O(3) bond, 1.242 Å, may be attributed to the strong hydrogen bond with N(6). The shortest C=O bond, C(6γ)–O(6δ), 1.210 Å, is the carbonyl of the ester linkage, and O(6δ) participates in only a weak hydrogen bond with N(3).

Small deviations from planarity can be observed in the peptide bonds and would be expected to reflect differences in the π-electron delocalization. The values of τ', χ_C, and χ_N are given in Table IV.[22] Usually in peptides where there is significant nonplanarity, the greatest out-of-plane bending occurs at the nitrogen atom, and such is the case for the methylproline–isoleucine, *N*-methylvaline–*N*-methylalanine, and *N*-methylalanine–β-alanine amide bonds. However, the nonplanarity of the isoleucine–*N*-methylvaline amide linkage is primarily attributable to a twist about the C′–N bond.

The ^{13}C NMR spectrum of roseotoxin B has been assigned previously.[2] The 250-MHz ^{1}H NMR spectrum of roseotoxin B (**1**) in chloroform gives sufficient chemical shift dispersion such that the majority of the protons appear as separated sets of resonances. Spin decoupling experiments at 250 MHz along with the previous 100-MHz data[2] allow an assignment of the spectrum as shown in Table V.

Previous studies have shown that the secondary structure of small polypeptides may be determined by NMR spectroscopy using either the temperature dependence of the NH proton chemical shifts, the deuterium–proton exchange rates, or solvent effects on the NH proton chemical shifts.[23,24,25] Comparison of the 250-MHz ^{1}H NMR spectra of roseotoxin B (**1**) in chloroform and methanol solutions shows that the β-alanine NH is shifted downfield in methanol by 0.3 ppm while the isoleucine NH proton remains essentially unchanged. This result indicates that the β-alanine NH proton is somewhat more exposed to the solvent than the isoleucine NH proton. This result is consistent with temperature dependency studies of a related compound[16] as well as with the X-ray structures reported in the present paper where the alanine NH is more accessible from the bottom face of the molecule shown in Figures 1 and 2.

Typical pathological signs of mice given a toxic dose of **1** were lethargy, tremors, hypermotivity, dyspnea, incoordination, and chronic convulsions.[26] The LD_{50} in day-old chickens was 12.5 mg/kg given orally.

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Registry No. Roseotoxin B, 55466-29-0; Destruxin A, 6686-70-0.

Supplementary Material Available: Tables of atom coordinates and temperature parameters for REH-RT, distances and angles for RB-RT and REH-RT, hydrogen atom parameters for REH-LT, anisotropic temperature parameters for RB-RT and REH-LT, Bijvoet differences (REH-LT), and structure factors for the three structures are available (52 pages). Ordering information is given on any current masthead.

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