10,10',12,12'-Tetrahydrosegoline B (18). Compound 18 was prepared in the same manner as described for 12 as an amorphous powder: ^{1}H NMR ($CDCl_3$) δ 8.62 (d, 1 H, J = 4.8 Hz, H-2), 7.86 (d, 1 H, J = 7.9 Hz, H-7), 7.40 (d, 1 H, J = 4.8 Hz, H-3), 7.30 (d, 1 H, J = 7.9 Hz, H-4), 6.92 (t, 1 H, J = 7.9 Hz, H-6), 6.97 (t, 1 H, J = 7.9 Hz, H-5), 6.78 (s, 1 H, H-14), 3.72 and 2.12 (AB quartet, 2 H, J = 12.2 Hz, H-10, 10'), 3.18 and 2.45 (an ABX, 2 H, J_{AB} = 11.6, J_{AX} = 2.0, J_{BX} = 1.5 Hz, H-12, 12'), 2.69 (d, 1 H, J = 2.0 Hz, H-13), 2.20 (s, 3 H, N(11)-Me), 1.88 (q, 1 H, J = 6.9 Hz, H-16), 1.69 (s, 3 H, Me-17), 0.74 (d, 3 H, J = 6.9 Hz, Me-18); EI MS, m/e (rel intensity) 371 (M^+, $C_{24}H_{25}N_3O$, 100).

Sodium Borohydride Reduction of 13 To Afford Compounds 19 and 20. Compounds 19 and 20 were prepared in the same manner as described for compounds 10 and 11.

19: an oil; ^{1}H NMR ($CDCl_3$) δ 8.70 (d, 1 H, J = 4.8 Hz, H-2), 7.97 (d, 1 H, J = 7.9 Hz, H-7), 7.52 (d, 1 H, J = 4.8 Hz, H-3), 7.40 (d, 1 H, J = 7.8 Hz, H-4), 7.33 (t, 1 H, J = 7.9 Hz, H-6), 6.95 (t, 1 H, J = 7.9 Hz, H-5), 6.87 (s, 1 H, H-14), 5.70 (s, 1 H, H-12), 4.05 (s, 3 H, Me-19), 3.63 (d, 1 H, J = 1.5 Hz, H-13), 2.97 (s, 3 H, N(11)-Me), 2.30 (dq, 1 H, J = 6.9, 1.5 Hz, H-16), 1.65 (s, 3 H, Me-17), 0.75 (d, 3 H, Me-18); EI MS, m/e (rel intensity) 401 (M^+, $C_{24}H_{23}N_3O_3$, 4), 149 (100).

20: an oil; IR ($CHCl_3$), 2900, 1660, 1580, 1400, 1280 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 8.13 (br s, 1 H, N(11)-H), 7.89 (d, 1 H, J = 4.8 Hz, H-2), 7.85 (d, 1 H, J = 7.9 Hz, H-7), 7.35 (d, 1 H, J = 4.8 Hz, H-3), 7.10 (d, 1 H, J = 6.9 Hz, H-4), 7.05 (t, 1 H, J = 7.9 Hz, H-6), 7.02 (t, 1 H, J = 6.9 Hz, H-5), 6.97 (s, 1 H, H-14), 4.47 and 4.12 (AB quartet, 2 H, J = 12.0 Hz, H-12, 12'), 3.92 (s, 3 H, Me-19), 2.84 (d, 3 H, Me-19), 2.84 (d, 3 H, J = 4.6 Hz, N(11)-Me), 2.76 (d, 1 H, J = 11.5 Hz, H-13), 2.41 (dq, 1 H, J = 11.5, 6.5 Hz, H-16), 1.63 (s, 3 H, Me-17), 1.25 (d, 3 H, J = 6.5 Hz, Me-18), (assignment of H-12 and H-12' was deduced from a COSY experiment); EI MS, m/e (rel intensity) 403 (M^+, $C_{24}H_{25}N_3O_3$, 4), 149 (100).

***N*(12)-Methylisosegoline A (21).** Compound 21 was prepared in the same manner as described for 8 as an amorphous powder: IR ($CHCl_3$) 2940, 1730, 1620, 1460 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 8.69 (d, 1 H, J = 4.8 Hz, H-2), 7.89 (d, 1 H, J = 7.9 Hz, H-7), 7.44 (d, 1 H, J = 4.8 Hz, H-3), 7.41 (t, 1 H, J = 7.9 Hz, H-6), 7.39 (d, 1 H, J = 7.9 Hz, H-4), 7.18 (s, 1 H, H-15), 5.08 (q, 1 H, J = 6.5 Hz, H-9), 4.07 (s, 3 H, Me-19), 3.72 (s, 1 H, H-14), 2.88 (s, 3 H, N(12)-Me), 1.49 (s, 3 H, Me-18), 1.16 (d, 3 H, J = 6.5 Hz, Me-17); CI MI, m/e (rel intensity) 399 (M^+ $C_{24}H_{21}N_3O_3$, 100).

***N*(1),*N*(8)-Dimethylnorsegoline (22).** Compound 22 was prepared in the same manner as described for 9 as an amorphous powder: IR ($CHCl_3$) 2940, 1680, 1620 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 9.40 (d, 1 H, J = 5.0 Hz, H-2), 8.34 (d, 1 H, J = 8.7 Hz, H-7), 8.15 (d, 1 H, J = 5.0 Hz, H-3), 8.03 (s, 1 H, H-10), 7.87 (t, 1 H, J = 8.7 Hz, H-6), 7.54 (d, 1 H, J = 8.7 Hz, H-4), 7.50 (t, 1 H, J = 8.7 Hz, H-5), 4.73 (s, 3 H, N(1)-Me), 4.08* (s, 3 H, OMe-11), 4.06* (s, 3 H, OMe-12), 3.70 (s, 3 H, N(8)-Me); CI MI, m/e (rel intensity) 335 (M^+, $C_{20}H_{19}N_2O_3$, 100).

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Registry No. 1, 117694-96-9; 2, 122795-54-4; 3, 117694-97-0; 4, 117694-98-1; 5, 122271-41-4; 6, 120154-96-3; 7, 122697-46-5; 8, 122697-47-6; 9, 122697-48-7; 10, 122697-49-8; 11, 122797-72-2; 12, 122697-50-1; 13, 122795-55-5; 14, 122795-56-6; 15, 122795-57-7; 16, 122697-51-2; 17, 122697-52-3; 18, 122795-58-8; 19, 122697-49-8; 20, 122697-53-4; 21, 122697-54-5; 22, 122697-55-6.

Conformational Properties of Ulithiacyclamide, a Strongly Cytotoxic Cyclic Peptide from a Marine Tunicate, Determined by ^{1}H Nuclear Magnetic Resonance and Energy Minimization Calculations

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The conformational properties of ulithiacyclamide, a highly cytotoxic cyclic peptide from an ascidian, have been investigated by usine one- and two-dimensional NMR spectroscopy, molecular modeling based on the NMR data, and molecular mechanics energy minimization. The temperature dependence of the NH protons and the nuclear Overhauser enhancements between the backbone and side-chain protons indicated that ulithiacyclamide, in its major form, assumes two kinds of conformations, which are highly dependent on the solvent. While the four NH bonds of the peptide linkages are all located in the interior of the ring structure in $CDCl_3$ and C_6D_6, two, which are related to each other by C_2 symmetry, protrude to the outer part of the ring structure in $(CD_3)_2SO$. Utilizing NMR parameters in conjunction with model building, extensive energy minimization calculations essentially led to two C_2 symmetric saddle-shaped conformations that best satisfied all of the NMR criteria. The two differed primarily in the direction of the NH bonds and the sign of the disulfide helicity. The sulfide linkage affects the molecular conformation of ulithiacyclamide.

Introduction

There is increasing interest in the biochemistry of marine tunicates because a high incidence of biological activity has been ascribed to their metabolites.[1] Lipophilic cyclic peptides that contain unusual amino acid moieties involving thiazole and oxazoline rings have been isolated from marine tunicates;[2] two representative structures are shown in Figure 1. These cyclic peptides, which exhibit potent cytotoxic activities,[3] all have a common or very

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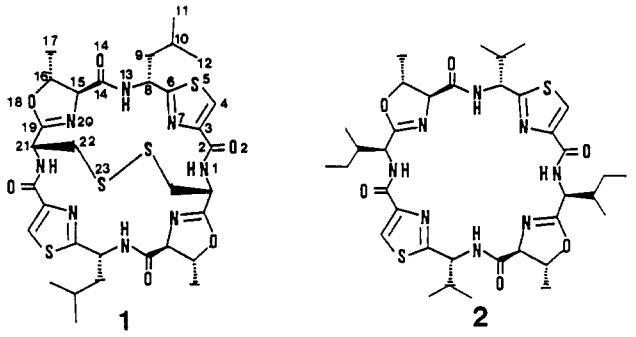

Figure 1. Chemical structures of representative cyclic peptide from ascidian: 1, ulithiacyclamide; 2, ascidiacyclamide. Atomic numbering used in this study is also shown in 1.

similar ring structure. This suggests a close relationship between ring conformation and the cytotoxic activity, although to date little is known about the mechanism of their biological action.

Ulithiacyclamide (1 in Figure 1), which was originally isolated and structurally characterized by Ireland and Scheuer[4] and Biskupiak and Ireland,[5] has a unique dimeric structure containing a disulfide bridge; the structure and absolute configuration were reassigned as the result of synthetic work.[6] Recent studies on structure–activity relationships have shown[7] that the high cytotoxic activity of ulithiacyclamide compared with the related cyclic peptides from tunicates is due to the presence of the oxazoline ring and the disulfide bond. Thus, we believe that a study of the molecular conformation of ulithiacyclamide and the effect of the oxazoline ring and disulfide bond on its conformation will provide the basic information needed to consider its biochemical function.

This paper deals with possible conformations of ulithiacyclamide on the basis of ^{1}H NMR studies, molecular modeling, and molecular mechanics energy minimization calculations. Previously, we reported the solution and solid-state conformations of ascidiacyclamide (2 in Figure 1).[8] Since this compound has the same ring structure as ulithiacyclamide, the structural parameters were helpful in molecular modeling of the latter molecule.

Results and Discussion

Ring Conformation of Ulithiacyclamide in Solution. All proton resonances were readily assigned by 2D COSY measurements; the assignments for $CDCl_3$ at 27 °C are shown in Figure 2. Similar ^{1}H NMR spectra were also obtained for C_6D_6 and $(CD_3)_2SO$ solutions. The peak assignments were essentially the same as those reported by Sesin et al.,[2] except for the D-Leu Cβ protons; a detectable chemical shift difference between these protons was observed in the present spectra, although they have been reported as multiplets. All resonances in the $CDCl_3$, C_6D_6, and $(CD_3)_2SO$ solutions showed that the dominant form of ulithiacyclamide had C_2 symmetry on the NMR time scale. Similar to the related cyclic peptides,[2,8b] homoallylic coupling between the H15 and H21 protons was observed. Thus, the occurrence of five-bond coupling through the oxazoline ring appears to be a general phenomenon.

Table I. NMR Parameters of NH Resonances

proton	solvt	δ (at 31 °C)	$-d\delta/dT \times 10^4$, ppm/°C
H1	$CDCl_3$	8.190	2.4
	C_6D_6	8.445	2.7
	$(CD_3)_2SO$	8.700	7.9
H13	$CDCl_3$	7.545	2.3
	C_6D_6	7.618	2.2
	$(CD_3)_2SO$	7.859	4.5

For estimating the entire ring structure of ulithiacyclamide, knowledge of the NH proton chemical shifts is very advantageous, because it is very sensitive to the environment. The temperature dependence of the amide proton chemical shift in various solvents has been used to distinguish between "exposed" and "buried" NH groups of peptides;[9] the exposed proton is affected more strongly than the buried one. The results for ulithiacyclamide are given in Table I. The characteristics of these amide protons can be summarized as follows: (a) the temperature coefficients ($-d\delta/dT$) of two amide protons are not significantly different and are within <0.003 ppm/°C in C_6D_6 and $CDCl_3$, while those in $(CD_3)_2SO$ exhibit a high temperature dependence (>0.004 ppm/°C), especially for the NH1 proton; (b) the rate of H–D exchange is very slow in $CDCl_3$ and C_6D_6 ($t_{1/2}$ = ca. 2 days, upon addition of methanol), while that in $(CD_3)_2SO$ shows a relatively short half-life ($t_{1/2}$ = ca. 18 min for H1 and 30 min for H13, upon addition of D_2O); (c) the NH13 resonance in the CCl_3 solvent shows a relatively small downfield shift with increasing concentration of $(CD_3)_2SO$ (7.761 ppm for 50% $CDCl_3$–$(CD_3)_2SO$), while the NH1 proton moves further downfield (8.449 ppm for a 50% mixture); (d) approximately 3-fold dilution of each solution causes no significant change in the chemical shifts of both amide protons. When the behavior of the NH protons is compared with that in related peptides,[8b,9,10] it appears that the ring conformation of ulithiacyclamide in $CDCl_3$ or C_6D_6 is different from that in $(CD_3)_2SO$. The $d\delta/dT$ values in $CDCl_3$ and C_1D_6 indicate that both NH protons are located in the interior of the ring structure. Generally, the $d\delta/dT$ value of the NH proton that is shielded from the solvent is on the order of 0–0.003 ppm/°C.[10a,f,11] The shielding of these NH protons from the solvent is also suggested from (b). On the other hand, the NH1 proton in $(CD_3)_2SO$ solution is characterized by a large $d\delta/dT$ value (a), a fast H–D exchange (b), and a large shift toward the downfield side by a polar solvent (c). These are typical features indicative of the exposure of the NH1 proton to the solvent. The NMR data for the NH13 proton in $(CD_3)_2SO$ show an intermediate behavior between the shielded and unshielded protons from the solvent molecule. The observation of (d) implies that the phenomena of (a)–(c) are due to the character of the ulithiacyclamide molecule itself rather

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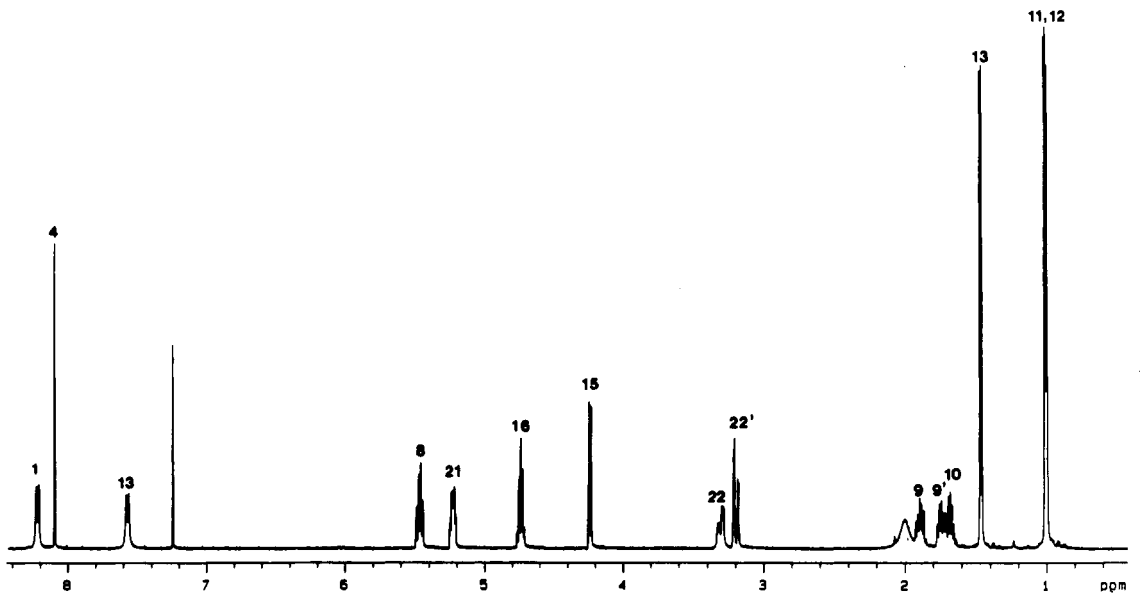

Figure 2. The 500-MHz ^1H NMR spectrum of ulithiacyclamide in $CDCl_3$ solution at 27 °C. The numbers correspond to those shown in Figure 1.

Table II. Possible Torsion Angles Estimated from $J_{NH-C_\alpha H}$ and $J_{C_\alpha H-C_\beta H}$ Coupling Constants at 31 °C

solvt	residue	$J_{NH-C_\alpha H}$, Hz	ϕ, deg	$J_{C_\alpha H-C_\beta H}$,[a] Hz	χ^1,[a] deg
$CDCl_3$	L-Cys	9.31	60, −112, −128	6.17	±9, ±81, ±111, ±159
	D-Leu	9.64	−60, 120	6.26	±21, ±81, ±99, ±159
C_6D_6	L-Cys	8.57	60, −100, −140	6.29	±9, ±82, ±111, ±158
	D-Leu	10.11	−60, 120	6.59	±11, ±84, ±109, ±156
$(CD_3)_2SO$	L-Cys	8.02	60, −94, −146	6.59	±11, ±84, ±109, ±156
	D-Leu	8.98	−60, 105, 135	5.93	±7, ±79, ±103, ±161

[a] The possible χ^1 angle was calculated by using the most reliable coupling constant between $J_{C_\alpha H-C_\beta H}$ and $J_{C_\alpha H-C_\beta H'}$ values. Thus, eight different values are possible.

than its molecular assembly.

As judged from the above-mentioned results, it is quite reasonable to imagine that the ring conformation of ulithiacyclamide predominantly assumes the type 1 form shown in Figure 3 in $CDCl_3$ or C_6D_6 solution and a type 2 form in $(CD_3)_2SO$ solution. As regards the latter form, the intramolecular N13–H···O2 hydrogen bond would be rather weak and readily broken by interaction with the solvent environment.

In addition to these NMR data of the amide protons, $J_{NH-C_\alpha H}$ and $J_{C_\alpha H-C_\beta H}$ coupling constants and ^1H NOE experiments further provide a variety of parameters that can be used to construct reasonable conformations of ulithiacyclamide in solution. The possible ϕ and χ^1 torsion angles, in which the notation corresponds to that of biochemical nomenclature,[12] of the L-Cys and D-Leu residues were estimated from the equations

$$J_{NH-C_\alpha H} = 7.9 \cos^2\theta - 1.55 \cos\theta + 1.35 \sin^2\theta \quad \text{(ref 13)}$$

$$J_{C_\alpha H-C_\beta H} = 11.0 \cos^2\theta - 1.40 \cos\theta + 1.6 \sin^2\theta \quad \text{(ref 14)}$$

where θ represents the torsion angle of the H–N–C_α–H or H–C_α–C_β–H bond sequence, respectively. The results are given in Table II. Among the torsion angles obtained from the calculations, a range of possible ϕ and χ^1 angles can

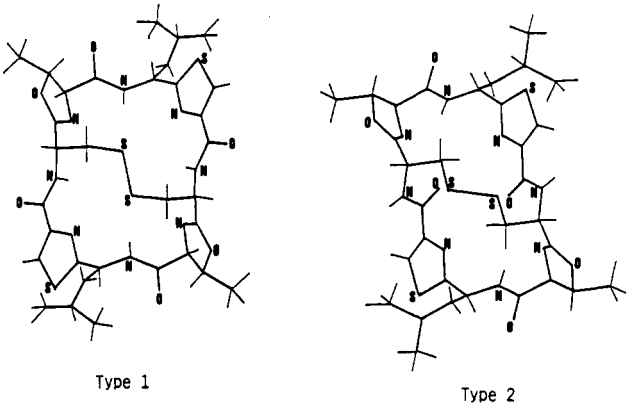

Figure 3. Schematic diagrams based on NMR data in $CDCl_3$ (type 1) and $(CD_3)_2SO$ (type 2) solutions.

Table III. ^1H NOE (percent) of Ulithiacyclamide in $CDCl_3$ and $(CD_3)_2SO$ Solutions at 31 °C

irradiation	proton (NOE[a])
	$CDCl_3$ Solution
H1	H21 (9.40), H22 (0.90)
H8	H9, 9′ (1.36), H10 (1.65), H11, 12 (1.04), H13 (2.82)
H13	H8 (6.23), H15 (7.18)
H15	H13 (2.90), H16 (5.05), H17 (0.93)
H21	H1 (2.47), H22 (3.80), H22′ (2.62)
	$(CD_3)_2SO$ Solution
H1	H4 (3.89), H21 (5.29)
H8	H9, 9′ (1.56), H10 (1.66), H11, 12 (0.82), H13 (2.19)
H13	H8 (4.30), H15 (4.72), H9, 9′ (1.51)
H15	H13 (2.14), H16 (2.91), H17 (1.92)
H21	H1 (2.24), H22 (2.82), H22′ (1.14)

[a] The value corresponds to the NOE per proton. The averaged error is 0.1%.

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Table IV. Estimated Torsion Angles (degrees) of Ulithiacyclamide in $CDCl_3$ and $(CD_3)_2SO$ Solutions[a]

residue		$CDCl_3$	$(CD_3)_2SO$
L-Cys	ϕ	−112, −128	60
	ψ	−30	−30
	ω	180	180
	χ^1	±9, ±81	±11, ±84
	χ^2	±130	±130
oxazoline	ϕ	120	120
	ψ	30	30
	ω	180	180
D-Leu	ϕ	120	105
	ψ	60	60
	ω	180	180
	χ^1	81, 99, ±159	79, 103, ±161
	χ^2	100	100
thiazole	ϕ	180	180
	ψ	0	180
	ω	180	180

[a] The definition of torsion angles for oxazoline and thiazole rings is as follows: oxazoline, ϕ = C19–N20–C15–C14, ψ = N20–C15–C14–N13, ω = C15–C14–N13–C8; thiazole, ϕ = C6–N7–C3–C2, ψ = N7–C3–C2–N1, ω = C3–C2–N1–C21′, where C21′ represents C21 related by C_2 symmetry.

be, to some extent, predicted by the rough conformation shown in Figure 3. However, more accurate information concerning the realistic ϕ and χ^1 values was obtained from the NOE experiments. From the NOE results, furthermore, we elicited information about the whole backbone and side-chain conformations, including ψ torsion angles of the L-Cys and D-Leu residues, because they reflect the spatial proximity of pairs of protons in solution. Among the proton pairs that showed strong interaction in the 2D NOESY experiments, the exact NOE values (in percent) were measured by 1D ^1H NOE experiments. The results in $CDCl_3$ and $(CD_3)_2SO$ solution are listed in Table III.

It is interesting to note that the stereochemical relationship among the H22 and H22′ atoms of L-Cys Cβ protons can be deduced from the NOE measurements. Irradiation of the H21 proton produced different NOEs for H22 and H22′ in $CDCl_3$ and $(CD_3)_2SO$ solution. This is very useful as it limits the χ^1 torsion angles of the L-Cys in Table II to a few values. On the other hand, the greater NOE of H10 as compared with those of H9,9′ and H11,12 produced by irradiation of the H8 proton yields information about χ^2 torsion angles of the D-Leu residue; the direction of the C8–H bond should be approximately parallel to that of the C10–H bond. Similarly, the NOE in H15 produced by irradiation of H13, which was somewhat larger than that in H8, gives a clue to the possible torsion angle of the oxazoline ring (ψ = N20–C15–C14–N13).

When the H1 proton was irradiated, an NOE was observed for the thiazole H4 proton in $(CD_3)_2SO$ solution. Since an NOE was not observed in $CDCl_3$ solution, this observation is in harmony with the ring conformation derived from the temperature dependence of the NH protons (see Figure 3): type 1 for the $CDCl_3$ solution and type 2 for the $(CD_3)_2SO$ solution.

Molecular Building of Ulithiacyclamide Solution Conformation. Combination of the above-mentioned NMR data permitted deduction of possible molecular conformations of ulithiacyclamide in solution. On the other hand, ascidiacyclamide has shown nearly the same temperature dependences as ulithiacyclamide in $CDCl_3$ and C_6D_6 solution,[8b] and its conformation has proved to be almost the same in solution and in the solid state.[8b] Therefore, the bonding parameters of its ring structure were used to construct the molecular conformation of ulithiacyclamide in $CDCl_3$ solution. The construction of the conformation in $(CD_3)_2SO$ solution was then derived from its conformation in $CDCl_3$.

Table V. Torsion Angles (degrees) and Intramolecular Hydrogen Distances (angstroms) of the Most Energetically Stable Conformations in $CDCl_3$ and $(CD_3)_2SO$ Solutions

		$CDCl_3$	$(CD_3)_2SO$
		Torsion Angle	
L-Lys	ϕ	−148.1	70.7
	ψ	−38.8	−44.5
	ω	−177.6	−166.5
	χ^1	58.9	−58.0
	χ^2	−89.4	151.7
oxazoline	ϕ	130.8	105.0
	ψ	−8.9	62.6
	ω	179.2	−173.9
D-Leu	ϕ	147.7	84.8
	ψ	60.6	54.6
	ω	−177.3	−177.1
	χ^1	173.1	174.2
	χ^2	79.4	83.1
thiazole	ϕ	175.7	176.3
	ψ	2.4	−178.1
	ω	−165.7	161.7
		$CDCl_3$	$(CD_3)_2SO$
		Distance	
H1–H4		4.57	1.99
H1–H21		2.95	2.15
H1–H22		3.43	4.07
H8–H9, 9′		2.52	2.47
		3.12	3.10
H8–H10		2.49	2.45
H8–H11, 12[a]		3.47	3.56
H8–H13		2.95	2.96
H13–H9, 9′		2.84	2.46
		3.23	2.66
H13–H15		3.42	2.55
H15–H16		3.07	2.89
H15–H17[b]		2.53	2.55
H21–H22		2.50	2.52
H21–H22′		2.51	3.10

[a] The shortest value among six different distances is listed. [b] The shortest value among three different distances is listed.

In building the model, the assumption was made that the peptide group neighboring the L-Cys residue is coplanar with the thiazole ring. This is based on the resonance effect in planar aromatic rings and has also been observed in the related ascidiacyclamide[8b] and nosiheptide molecules.[15] The possible torsion angles built up by the conjunction with space-filling models and the NMR parameters are given in Table IV. The major difference between the conformations in the $CDCl_3$ and $(CD_3)_2SO$ solutions is in the ϕ(L-Cys) and ψ(thiazole) torsion angles.

Conformational Refinement by Energy Minimization. To obtain more realistic and reliable solution conformations than those listed in Table IV, the energetically stable conformations were surveyed by an energy minimization technique using a molecular mechanics program, MMFF. All combinations of torsion angles listed in Table IV (64 sets for the $CDCl_3$ solution and 32 sets for the $(CD_3)_2SO$ solution) were used as the starting conformations for energy minimization, and the resulting conformations were examined for their compatibility with the NMR parameters.

It is interesting to note that all of the starting conformers more or less converged into two kinds of conformations, which have been classified as types 1 and 2 in Figure 3. The most energy-refined torsion angles for the conformations are given in Table V, and their stereoscopic views are shown in Figures 4 and 5. These conformations agree

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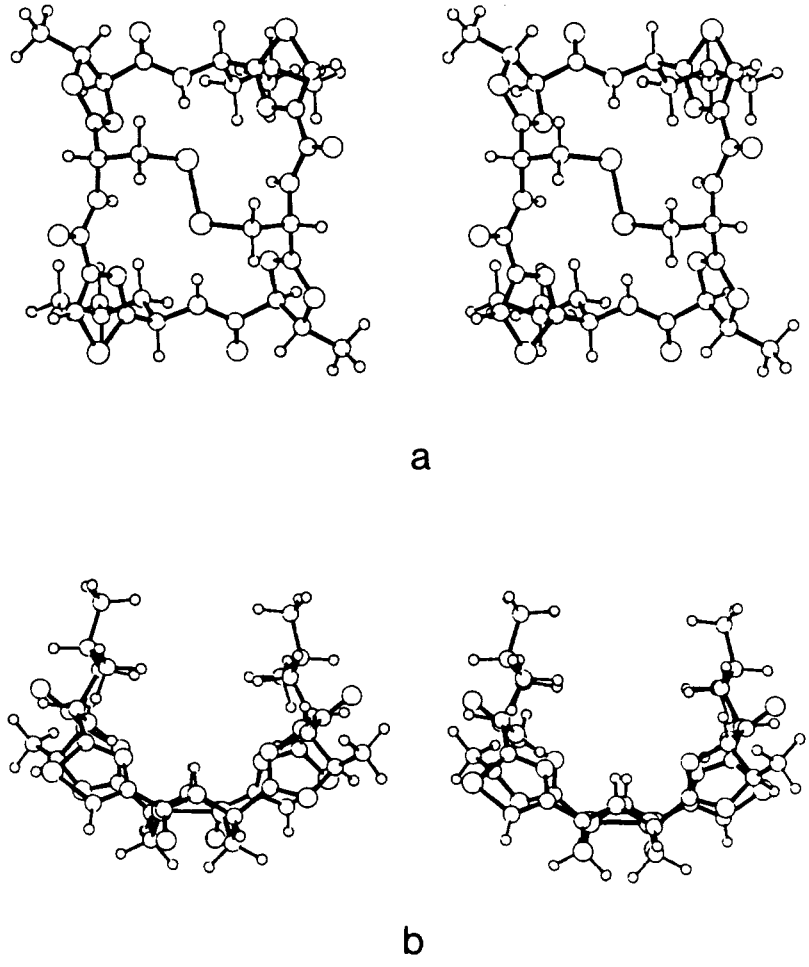

Figure 4. Stereoscopic views of ulithiacyclamide proposed as the most stable conformation in $CDCl_3$ solution, viewed along the C_2 symmetry axis (a) and perpendicular to this axis (b).

well with the NMR parameters; as is shown in Table V, the proton pairs showing large NOE values are all within a short distance (<4.0 Å), and the C_2 symmetry of the molecule itself is maintained.

The characteristics of these conformations can be described as follows. For the type 1 conformation in $CDCl_3$ solution (see Figure 4), the ring structure is cylindrically curved with the top line joining two S23 atoms related by C_2 symmetry. The radius of the curvature is about 3.4 Å. The peptide group adjacent to the L-Cys is almost coplanar with the thiazole ring and forms a slightly warped plane between the thiazole and oxazoline rings. The ring structure as a whole assumes a rectangular shape with a long axis of ca. 8 Å and a small axis of ca. 6 Å. The type 2 conformation, however, which corresponds to the form in $(CD_3)_2SO$ solution (see Figure 5), slants toward the corner at which the oxazoline ring is located. The angle between the directions of the C6–C15 and C19–C3′ bonds is 76°. Thus, the width between the two oxazoline–L-Cys–thiazole backbone chains related by C_2 symmetry becomes narrower by ca. 1 Å as compared with the type 1 conformation. As with the type 1 conformation, this ring structure also forms a cylindrically curved conformation. As is obvious from the comparison of Figure 5b with Figure 4b, however, the bottom of the former curvature is rather shallow, and the side is considerably wider (the radius of the curvature = ca. 4.5 Å).

The essential difference between the conformations of type 1 and type 2 is in the thiazole ψ, oxazoline ψ, and L-Cys ϕ, χ^1, and χ^2 torsion angles. While four NH bonds in the type 1 conformation are all directed toward the interior of the ring structure, two NH bonds, which are adjacent to the thiazole rings, are directed away from the ring structure in the type 2 conformation. Furthermore, the difference in the L-Cys χ^1 and χ^2 torsion angles converts the left-handed helicity (type 1) of the disulfide linkage to a right-handed one (type 2). Since the molecular conformations shown in Figures 4 and 5 have nearly the same total energy (−12.5 kcal/mol for type 1 and −11.8 kcal/mol for type 2), it may be that interconversion between these conformations can occur quite readily upon an environmental change such as a solvent, as actually observed in the NMR experiments.

The total energy for each conformer is mainly determined by van der Waals interactions. The contribution of intramolecular hydrogen bonds was not important; the distance between the N13 and O2 atoms in the type 2 conformation was 5.004 Å. This is in agreement with the relatively large $d\delta/dT$ value of the H13 atom (see Table I).

As stated in the Introduction, many lipophilic cyclic peptides isolated from marine tunicates exhibit potent antineoplastic and/or cytotoxic activities. Among them, ulithiacyclamide possesses the highest activity. Since the activity is very likely related to the molecular conformation, it is important to know the actual spatial form for considering the biological function. Two kinds of major conformations were elucidated for ulithiacyclamide by NMR and energy minimization studies; these conformations were highly dependent on the character of the solvent. The type 1 conformation was the predominant form in a relatively nonpolar solvent such as C_6H_6 or $CHCl_3$,

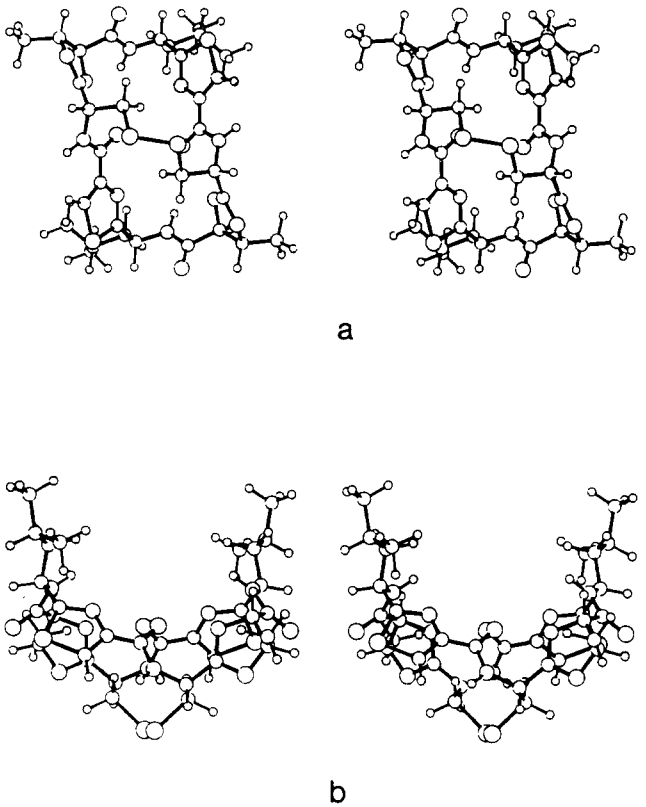

Figure 5. Stereoscopic views of ulithiacyclamide proposed as the most stable conformation in the $(CD_3)_2SO$ solution, viewed along the C_2 symmetry axis (a) and perpendicular to this axis (b).

whereas the type 2 conformation predominates in polar solvents, such as $(CH_3)_2SO$ and probably H_2O.[16]

It is of interest that the type 1 conformation of ulithiacyclamide is very similar to the solution and solid-state conformations of ascidiacyclamide;[8] the latter molecule, in its major form, has a type 1 conformation irrespective of the solvent. This was somewhat surprising because formation of the disulfide bridge in ulithiacyclamide was thought to confer additional rigidity to the ring conformation. Judging from the present results, however, we may conclude that the presence of the disulfide bond in ulithiacyclamide lends greater flexibility to the molecular conformation; in other words, t[illegible] conformational adaptability is caused as a result of t[illegible] disulfide linkage. The oxazoline ring appeared to be important for removing any constraint produced by the conformational change. Compared with the type 1 conformation of ulithiacyclamide, the type 2 conformation provides a more accessible interface with which a bimolecule may interact. There is also a characteristic difference between the spatial arrangements of the polar atoms within the ring structure; while in the type I conformations the NHs of peptides and the Ns of the thiazole and oxazoline rings, representing the donor and acceptor atoms, respectively, are alternately arranged around the ring structure at nearly the same intervals, the interior of the type 2 ring conformation is fully occupied by the acceptor atoms, except for two N13–H bonds. Although the detailed mechanism by which ulithiacyclamide exerts its biological action requires further study, the conformational flexibility and different arrangement of the polar atoms, which would be important for the interaction with biomolecules, may be closely related to its biological activity.

(16) Ulithiacyclamide is insoluble in water.

Experimental Section

Materials. Ulithiacyclamide was chemically synthesized according to ref 6a. The purity was checked by the elution profile of high-pressure liquid chromatography and the ^1H NMR method. All other materials used were commercial preparations (reagent grade) and were used without further purification.

^1H NMR Measurements. ^1H NMR experiments were conducted on a Varian XL-200, XL-300, or XL-500 NMR spectrometer. The sample concentration was gravimetrically adjusted to ca. 7.6 mg/0.5 mL (~20 mM). The solvents used were $CDCl_3$, C_6D_6, and $(CD_3)_2SO$ (each of 99.5% isotopic purity). The proton chemical shifts (δ) were measured as a downfield shift from internal tetramethylsilane (TMS); the margins of error were within ±0.001 ppm and ±0.1 Hz.

The temperature dependence of the amide proton resonances was determined by six measurements over a range of 20–70 °C for $(CD_3)_2SO$, 10–50 °C for $CDCl_3$, and 10–60 °C for C_6D_6 solution by using a saturating pulse at TMS resonance; the temperature-controlled unit has an error within ±1 °C. For the nuclear Overhauser enhancement (NOE) experiments, the sample solutions were completely degassed with four freeze–pump–thaw cycles directly into the NMR tubes, which were then sealed.

One-dimensional ^1H NOE difference spectra were acquired automatically by using both on- and off-resonance spectra. In the experiments, a series of decoupler frequencies were chosen, one for each line, four lines at maximum for the multiplet to be irradiated, and the decoupler was cycled through this series of frequencies during the irradiation period (10–20 s), resulting in efficient saturation at low decoupler power levels. Then data acquisition occurred with the decoupler gated off. Each free irradiation decay (FID) corresponding to each irradiation was acquired. Difference spectra were obtained in the FID stage prior to the Fourier transformation by the subtraction of the control (off-resonance irradiation) from every other spectrum. The total number of scans acquired for each spectrum was typically 400.

Two-dimensional correlated spectroscopy (COSY) experiments employed a spectral width of ca. 4000 Hz in both columns ω_1 and ω_2. A total of 1024 points were recorded in t_2, 128 points in t_1 with zero filling to 1024. A total of 128 increments was used for t_1. The pulse sequence used was t_0–90°(ϕ_1)–t_1–90°(ϕ_2)–FID, where t_0 is the pulse delay, and t_1 is the evolutionary time. Two-dimensional ^1H NOE spectroscopy (NOESY) experiments were performed using the States-Haberkorn method (2D hypercomplex method).[17] FIDs were acquired (48 scans, 8 dummy scans) over 4000 Hz into a 2K data block for 512 incremented values of the evolution time t_1. The raw data were zero-filled to a 2K × 2K matrix. The mixing time (t_m) of 0.45 s was used, and the relaxation delay, D_1, was 2.0 s. The applied pulse sequence was t_0–90°(ϕ_1)–t_1–90°–(ϕ_2)–t_m–90°(ϕ_3)–FID.

Energy Minimization Calculations. All energy calculations were carried out with the molecular mechanics force field (MMFF) program[18] within the CHEMLAB-II system[19] operating on a MicroVAX II computer. The empirical energy functions used to obtain the energy-minimized structures included harmonic potential energy terms for bond lengths, bond angles, bond dihedral angles, torsion angles, and van der Waals and electrostatic terms for nonbonded interactions and a hydrogen bonding potential. The computational methodology and the general forms of the potential functions correspond to those described by Pearlstein.[20]

The atomic coordinates of the ulithiacyclamide ring structure were constructed by using the bond lengths and angles of ascidiacyclamide evaluated by the X-ray analysis.[8b] The L-cystine moiety was constructed by using the X-ray data of the L-cystine crystal[21] in which the molecule takes an approximate C_2 symmetry

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between the molecule halves. Solvent molecules were not included in the calculations, but the effect was taken into account by employing a dielectric constant of 5.0 for $CHCl_3$ and 78.0 for $(CH_3)_2SO$;[22] the value for $(CH_3)_2SO$ corresponds to that for H_2O.

The starting conformations for energy minimization calculations were built up by using the possible torsion angles estimated by NMR experiments. Any structural constraints imposed while building plausible starting conformations were relaxed during the calculation, and the energy minimization was allowed to proceed freely without any conformational constraints. The refined conformers that were not consistent with the NMR results were rejected from the consideration.

Registry No. 1, 74847-09-9.

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Structure and Absolute Configuration of Spathulasin, a Metabolite of *Aeonium spathulatum*

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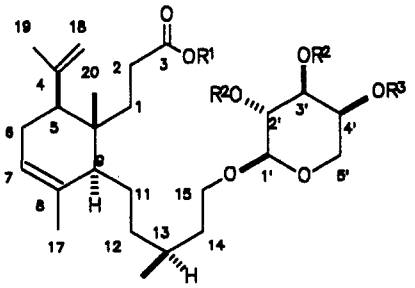
A new metabolite, spathulasin (1), is described as a natural product from *Aeonium spathulatum*. The structure of the 14-membered lactone derivative **10** was determined by X-ray crystallographic analysis. Hydrolysis of spathulasin and identification of the pentose portion as L-arabinose allowed the assignation of the absolute configuration of all centers in **1**.

Among the genera of Crassulaceae, *Aeonium*, which is developed to a striking degree in the Canary Islands,[1] has received scarce attention from the chemical point of view.[2] We have investigated the constituents of *Aeonium spathulatum* (Hornem.) Praeger and herein describe the isolation and structure determination of a novel diterpene glycoside.

Spathulasin (1) was isolated from the alcoholic extract of *A. spathulatum* as an amorphous solid. Data from



1 $R^1 = R^2 = H$, $R^3 = (CH_3)_2C{=}CHCO$

2 $R^1 = Me$, $R^2 = H$, $R^3 = (CH_3)_2C{=}CHCO$

3 $R^1 = H$, $R^2 = Ac$, $R^3 = (CH_3)_2C{=}CHCO$

4 $R^1 = R^2 = R^3 = H$

5 $R^1 = Me$, $R^2 = R^3 = H$

high-resolution mass and ^{13}C NMR spectrometry established a molecular formula of $C_{30}H_{48}O_8$. The IR spectrum showed the presence of a carboxylic acid (3500–2400 and 1700 cm^{-1}) and a methylenic double bond (1640 and 895 cm^{-1}). The ^1H NMR spectrum (Table I) exhibited resonances that could be assigned to six methyl groups, one singlet at δ 0.81, one doublet at δ 0.89 (J = 5.8 Hz), and four wide singlets at δ 1.68, 1.76, 1.87, and 2.13 that indicated vinylic methyls. Furthermore, observation of the δ 4–5 region, together with the number of oxygens present in the molecular formula, strongly suggested that spathulasin (1) contains a sugar moiety. Six ^{13}C NMR resonances at δ 114.02 (t), 115.86 (d), 121.96 (d), 136.15 (s), 147.80 (s), and 158.49 (s) pointed out the presence of three olefinic bonds in the molecule, a signal at δ 103.18 (d) was attributed to an anomeric sugar carbon, and two downfield signals at δ 166.47 (s) and 178.71 (s) were assigned to carboxylic groups.

Methylation of spathulasin (1, excess diazomethane in diethyl ether) yielded methyl ester **2** in high yield. The spectroscopic data of **2** confirm the aforementioned structural features.

Treatment of spathulasin (1) with excess acetic anhydride in pyridine at room temperature afforded diacetate **3** as shown by its ^1H NMR spectrum where two new methyl resonances at δ 2.00 and 2.05, corresponding to the esters formed, were observed.

Basic hydrolysis of spathulasin (1) gave **4**, which was characterized as its methyl ester **5**. From high-resolution mass spectrometry it was evident that the esterifying acid had a molecular formula of $C_5H_8O_2$. Comparison of the ^1H NMR spectra of **1** and **5** showed that the latter lacks the low-field methyl groups at δ 1.87 and 2.13 and the olefinic proton at δ 5.75. Also when the ^{13}C NMR spectra of these compounds were compared, the absence in **5** of five carbons at δ 20.56 (q), 27.60 (q), 115.86 (d), 158.49 (s), and 166.47 (s) was observed. These results indicate that the esterifying acid is 3,3-dimethylacrylic (senecioic) acid.[3]

Acid hydrolysis of methyl ester **5** produced cleavage of the sugar moiety to yield aglycon **6**. High-resolution mass

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