That the controlled-potential electrolysis produced the monoprotonated form DH^+ is evidenced by the two following results: (i) UV–visible spectrometry in aqueous media indicates that the first protonation of D occurs with a pK_a of ca. 0.6 to give DH^+. On the basis of these spectral changes the spectrum of D in ACN (1.05 M $HClO_4$) corresponds to that of the only acidic species DH^+; (ii) HPLC analysis of a solution of DH^+ prepared in ACN (1.05 M $HClO_4$) and chromatographed under the same conditions as those used for the electrolyzed solution of 5HT reveals the presence of only one HPLC peak due to D; i.e., the buffer capacity of the HPLC eluent is efficient enough to convert remaining DH^+ to D.

Formal Kinetics. The rsc mechanism consists of the reactions given in eq 2–5 (see text). In the present case, reaction 5 is practically irreversible since DH^+ is the only major product that exists at equilibrium after electrolysis. The stationary-state approximation is assumed concerning $DH_2^{\bullet+}$ and DH_2^{2+}. Deprotonation (eq 5) is the rds if $k_5 \ll k_{-4}C_{AH}$ and at the same time $k_{-3} \gg K_4k_5C_{AH^{\bullet+}}/C_{AH}$. It follows that the apparent rate constant is $k_{app} = 2K_3K_4k_5$; then $E_{pa} = E^\circ + 50.6 - 19 \log K_3K_4k_5Cv^{-1}$ and $E_{pa} - E_{pa/2} = 37.5$ mV, the potentials being expressed in mV and the temperature being 15 °C.

The rrc mechanism would consist of the reactions given in eq 2 (see text), 6, and 7:

$$AH^{\bullet+} \underset{k_{-6}}{\overset{k_6}{\rightleftharpoons}} A^\bullet + H^+ \qquad K_6 = k_6/k_{-6} \tag{6}$$

$$A^\bullet + AH^{\bullet+} \xrightarrow{k_7} DH^+ \tag{7}$$

The stationary-state approximation can be performed by treating $A^\bullet$ as a reactive intermediate. Coupling 7 is the rate-determining step (rds) as soon as $k_{-6}C_{H^+} \gg k_7C_{AH^{\bullet+}}$. Now the apparent rate constant becomes $2K_6k_7/C_{H^+}$; then $E_{pa} = E^\circ + 50.6 - 19 \log K_6k_7C/C_{H^+}v$ and $E_{pa} - E_{pa/2} = 37.5$ mV.

Acknowledgment. We acknowledge support of this work by the CNRS (Unité Associée 484 "Synthèse et électrochimie des composés d'intérêt pharmacologique"). J. P. Seguin and G. Goethals (Université de Picardie, Amiens, France) are thanked for running the NMR spectra.

Supplementary Material Available: One figure giving the HPLC chromatogram of the reaction mixture resulting from the electrolysis of 5HT in acidic ACN, and the mathematical treatments of the formal kinetics (4 pages). Ordering information is given on any current masthead page.

Structure of FR900359,[1] a Cyclic Depsipeptide from *Ardisia crenata sims*

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Received December 1, 1986

The structure of FR900359, a novel cyclic depsipeptide from *Ardisia crenata sims* that shows inhibition of platelet aggregation and decrease of blood pressure, has been determined as 3-acetamido-22-benzyl-10-[1-[(3-hydroxy-4-methyl-2-propionamidopentanoyl)oxy]-2-methylpropyl]-4-isopropyl-7-(1-methoxyethyl)-19-methylene-8,13,14,16,20-pentamethyl-1,5-dioxa-8,11,14,17,20-pentaazacyclodocosane-2,6,9,12,15,18,21-heptone by hydrolytic, ^1H and ^{13}C nuclear magnetic resonance spectroscopic, and mass spectrometric studies. FR900359 consists of ten units: alanine, *N*-methylalanine, β-hydroxyleucine (three residues), 3-phenyllactic acid, acetic acid, propionic acid, and the uncommon amino acids *N*-methyldehydroalanine and *N,O*-dimethylthreonine.

Introduction

Ardisia crenata sims is an evergreen plant that grows abundantly in the Far East and is widely used as an ornamental plant in Japan. As a result of the continuing search for potentially therapeutic materials in natural products, we found that a cyclic depsipeptide, code-named FR900359, isolated from a methanol extract of the whole plants of *Ardisia crenata sims*, inhibits platelet aggregation in rabbits in vitro, decreases the blood pressure, and causes dose-related hypotension in anesthetized normotensive rats.[3] It is cytotoxic to cultured rat fibroblasts and myelocytic leukemia cells.

We report the structure determination of FR900359.[4] Of particular significance of this cyclic depsipeptide is the uncommon amino acid *N*-methyldehydroalanine found previously only in a toxin from the blue-green alga *Mi-*

Table I. ^1H and ^{13}C NMR Spectral Data of FR900359

^1H chemical shift,[a,b] δ	nature of hydrogens
0.8–1.4 (m,[c] ca. 30)	aliphatic methyl
1.9–5.4 (m,[c] ca. 20)	methylene, methine
2.21 (s, 3), 2.67 (s, 3), 2.87 (s, 3), 3.14 (s, 3), 3.39 (s, 3)	*O*-methyl, *N*-methyl, acetyl
6.74 (d, 1), 6.84 (d, 1), 7.12 (d, 1), 7.56 (d, 1), 8.50 (d, 1)	amide CH–NH–CO, alcohol CH–OH
7.28 (s, 5)	aromatic hydrogen
^{13}C chemical shift,[d] δ	**nature of carbons[e]**
10–73 (ca. 30)	methyl, methylene, methine
106.7 (1)	olefinic $=CH_2$
126.9 (1), 128.5 (2), 129.6 (2)	aromatic =CH
136.0 (1), 145.3 (1)	aromatic =C, olefinic =C
163.8 (1), 166.4 (1), 167.7 (1), 169.2 (1), 169.9 (1), 170.2 (1), 171.2 (1), 171.3 (1), 172.4 (1), 174.7 (1)	amide CO, ester CO

[a] Multiplicity: s; singlet, d; doublet, m; multiplet. [b] Number of hydrogens. [c] Assignments of these signals were difficult, because of their insufficient splitting. [d] Number of carbons. [e] Nature of carbons was proposed by the chemical shift values and proton off-resonance experiments.

(1) FR900359 for this cyclic depsipeptide is a code number used by Fujisawa Pharmaceutical Co., Ltd.

(2) Most of this work has been carried out at the Department of Chemistry, Massachusetts Institute of Technology, during a sabbatical leave (M.F.) from Analytical Research Laboratories, Fujisawa Pharmaceutical Co., Ltd.

(3) Horiai, H.; Shimizu, I.; Shibayama, F.; Kikuchi, H., unpublished results.

(4) A preliminary investigation of the structure of FR900359 had been carried out in collaboration with Masataka Shigi, Keiichi Nakashima, Isamu Sumino, and Sueo Atarashi at Analytical Research Laboratories, Fujisawa Pharmaceutical Co., Ltd.

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crocystis aeruginosa[5] and the novel amino acid *N,O*-dimethylthreonine.

Results and Discussion

FR900359 was found to have the molecular formula $C_{49}H_{75}N_7O_{15}$ on the basis of a molecular weight of 1001 determined by fast atom bombardment (FAB) and electron impact (EI) mass spectrometry (MS) and combustion analyses. This formula was also confirmed by high-resolution (HR) EI mass measurements. ^1H and ^{13}C NMR data contained signals for about 75 hydrogens and 50 carbons, which were assigned as shown in Table I. The IR spectrum showed a characteristic ester carbonyl band at 1750 cm^{-1} and amide carbonyl bands at 1665 and 1635 cm^{-1}. The ten carbonyl carbons assigned in the ^{13}C NMR spectrum are, therefore, attributed to amide and ester groups. The observations that FR900359 was ninhydrin-negative and did not react with diazomethane implied that FR900359 had neither free carboxylic acid nor free amino groups. These data suggested that FR900359 was a cyclic depsipeptide composed of ten acid units; based on the molecular formula, at most seven of them must be amino acids and the remaining ones are presumed to be hydroxy acids.

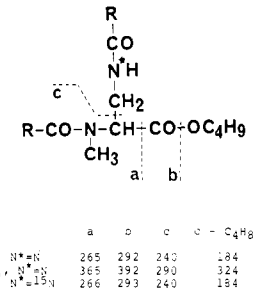
Complete hydrolysis with constant boiling hydrochloric acid produced alanine, *N*-methylalanine, β-hydroxyleucine, and methylamine (1.0:1.0:2.2:1.2, by molar ratio) identified by amino acid analysis and 3-phenyllactic acid identified by gas chromatography–mass spectrometry (GC/MS), which also confirmed the three amino acids above as their *N*- and *O*-trifluoroacetyl butyl ester derivatives.

β-Hydroxyleucine was found not to be very stable under the hydrolytic condition, giving 4-methyl-2-oxopentanoic acid in the hydrolyzate of authentic β-hydroxyleucine. The formation of 2.2 mol of β-hydroxyleucine in the hydrolyzate of FR900359 suggests, therefore, that FR900359 contains at least three β-hydroxyleucine residues.

The presence of an *N*-methyldehydroamino acid in FR900359 was suggested by the formation of methylamine during the acid hydrolysis of FR900359. The other acidic degradation product to be expected from *N*-methyldehydroamino acid is an α-keto acid and this was identified as the trimethylsilylated quinoxalinol derivative[6] by treatment of the hydrolyzate of FR900359 with 1,2-phenylenediamine followed by trimethylsilylation. The derivatives of pyruvic acid, 4-methyl-2-oxopentanoic acid and 2-oxobutyric acid (trace amount) were identified. 4-Methyl-2-oxopentanoic acid is the expected degradation product of β-hydroxyleucine, and the formation of pyruvic acid and methylamine indicates the presence of *N*-methyldehydroalanine. Further evidence for the presence of an *N*-methyldehydroalanine residue was obtained by reduction of FR900359 with sodium borodeuteride, followed by complete hydrolysis affording *N*-methylalanine-*d*.

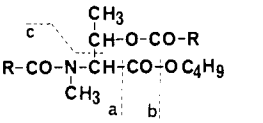
Gas chromatography (GC) of the hydrolyzate revealed that acetic acid and propionic acid are formed in an approximate 1:1 molar ratio.

The acid hydrolysis products described above implied that the following nine units were constituents of the



	a	b	c	a − C4H8	a − C4H9OH
2 : R=CF3, N*=N	265	292	240	184	167
3 : R=C2F5, N*=N	365	392	290	324	217
6 : R=CF3, N*=15N	266	293	240	184	167

Figure 1. Fragmentation (EI) of the butyl ester of acetylated 3-amino-2-(methylamino)propionic acid (**2**), the butyl ester of pentafluoropropionylated 3-amino-2-(methylamino)propionic acid (**3**), and the butyl ester of acetylated 3-(^{15}N)amino-2-(methylamino)propionic acid (**6**).



	a	b	c	a − RCOOH	a − C4H8	M − H2O
4 : R=CF3	280	308	240	166	184	363
5 : R=C2F5	390	408	290	216	234	463

Figure 2. Fragmentation (EI) of the butyl ester of acetylated *N*-methylthreonine (**4**) and the butyl ester of pentafluoropropionylated *N*-methylthreonine (**5**).

FR900359 molecule: alanine, *N*-methylalanine, *N*-methyldehydroalanine, β-hydroxyleucine (three residues), 3-phenyllactic acid, acetic acid, and propionic acid. If connected by amide and ester bonds to form a cyclic depsipeptide, they account for $C_{43}H_{64}N_6O_{13}$, leaving $C_6H_{11}NO_2$ for the tenth unit, to complete the molecular formula of FR900359. The nine units account for most of the NMR data, and the remaining NMR signals indicated that there should be two methyl groups attributable to *N*-methyl and *O*-methyl, one aliphatic methyl (from the ^1H NMR), and one carbonyl carbon but no olefinic carbon (from the ^{13}C NMR) in the $C_6H_{11}NO_2$ moiety. The complete structure of the tenth unit of FR900359 was, however, not readily apparent at this point. However, this moiety was later identified as *N,O*-dimethylthreonine in the course of the investigation of an ammonolysis product (**1**) isolated upon treatment of FR900359 with 20% aqueous ammonia–methanol (1:1) for 48 h at room temperature. The structure of **1** was deduced from the following experiments.

The FAB mass spectrum of **1** exhibited a $(M + H)^+$ ion at *m/z* 681 and a number of abundant fragment ions. Acid hydrolysis of **1** with constant boiling hydrochloric acid produced alanine, *N*-methylalanine, β-hydroxyleucine, 3-phenyllactic acid, and two other compounds. These were shown to be 3-amino-2-(methylamino)propionic acid and *N*-methylthreonine, on the basis of the mass spectra of their *N*-perfluoroacyl *O*-butyl esters (Figures 1 and 2, respectively). That the 3-amino-2-(methylamino)propionic acid is the product of ammonia addition to the β-carbon of the *N*-methyldehydroalanine residue was demonstrated by the identification (by GC/MS) of 3-(^{15}N)amino-2-(methylamino)propionic acid in the hydrolyzate of **1**(^{15}N), produced by treatment of FR900359 with methanolic (^{15}N)ammonia under the conditions described above.

The structure of **1** (Figure 3) was deduced from its FAB mass spectrum with the aid of those of the corresponding N,O-acetylated (CH_3CO and CD_3CO, respectively) methyl esters **7** and **8** and of ^{15}N-labeled **1**. Exact mass measurements of the ions of *m/z* 681 and 534 in the spectrum of **1** revealed the compositions $C_{32}H_{53}N_6O_{10}$ and $C_{26}H_{40}$-

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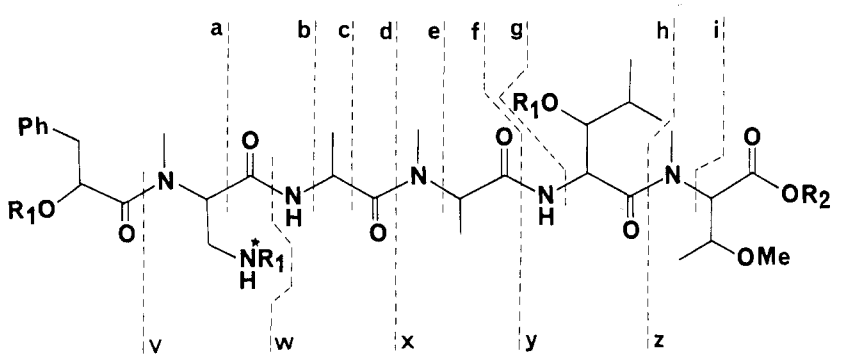
(9) Liesch, J. M.; Millington, D. S.; Pandey, R. C.; Rinehart, K. L., Jr. *J. Am. Chem. Soc.* **1976**, *98*, 8237.

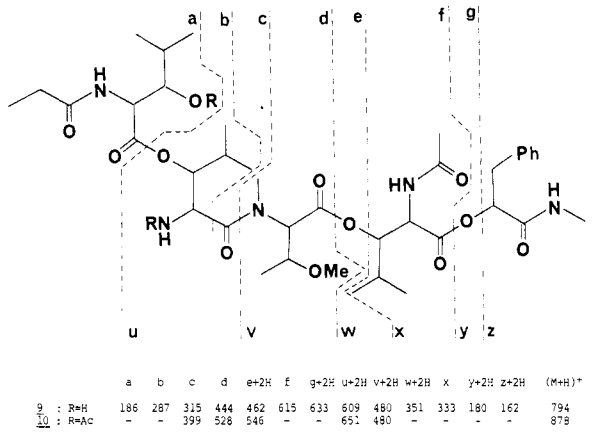


		a	b+2H	c	d	e+2H	f	g+2H	h	i+2H	v+2H	w+2H	x+2H	y+2H	z+2H	$(M+H)^+$
1	: $R_1=R_2=H$, $N^*=N$	221	266	292	320	351	405	422	534	565	533	433	362	277	148	681
1(^{15}N)	: $R_1=R_2=H$, $N^*=^{15}N$	222	267	293	321	352	406	423	535	-	534	433	362	277	148	682
7	: $R_1=Ac$, $R_2=Me$, $N^*=N$	305	-	-	404	435	489	-	660	-	-	489	418	-	162	821
8	: $R_1=Ac(d_3)$, $R_2=Me$, $N^*=N$	311	-	-	410	441	495	-	669	-	-	-	-	-	162	830

Figure 3. Structures of 1, 7, and 8 with an indication of the fragment ions observed in their FAB mass spectra.

N_5O_7, respectively. Acetylation indicated the presence of a total of three amino or hydroxyl groups. According to the 42 x n dalton shifts in the mass spectra, two of these must be located within the first two and one at the fifth unit. Only one esterifiable carboxyl group is present and it must be at the C-terminus. When **1** was produced from FR900359 by ammonolysis with $^{15}NH_4OH$, the mass of all N-terminal fragment ions and of $(M + H)^+$ increased by 1 dalton, indicating that one nitrogen atom is incorporated in the course of the ammonia treatment and that it is located in the second unit. Needless to say, the interpretation of these FAB mass spectra were greatly facilitated by the knowledge of the structure of the six compounds produced upon acid hydrolysis as outlined earlier. The sum of their elemental compositions minus $5 \times H_2O$ corresponds to $C_{31}H_{50}N_6O_{10}$, which is CH_2 less than the composition of **1**. Since the fragment of m/z 534 represents the loss of $C_6H_{13}NO_3$ which must be from the C-terminus (based on the C/H ratio alone), the additional CH_2 must reside there in the form of a homologue or methyl derivative. The only apparent discrepancy is the finding of *N*-methylthreonine in the hydrolyzate, instead of *N,O*-dimethylthreonine. This conversion can be rationalized by β-elimination of methanol to 2-(methylamino)-2-butenoic acid which adds water to form *N*-methylthreonine. This possibility is substantiated by the finding of 2-oxobutyric acid, in addition to 4-methyl-2-oxopentanoic acid (from β-hydroxyleucine) as well as methylamine when the acid hydrolyzate of **1** was searched for keto acids. It is also surprising that **1** has a free carboxyl group rather than an amide at the C-terminus. The most likely explanation is hydrolysis during the evaporation of the acidic (TFA) eluent of the HPLC fraction.

Assembling the ten units into the complete structure of FR900359 is based on the ammonolysis data mentioned above and on the mass spectral data of compounds produced upon partial acid hydrolysis and methanolysis of FR900359. *N*-Acetyl-β-hydroxyleucine, *N*-propionyl-β-hydroxyleucine, alanyl-*N*-methylalanyl-β-hydroxyleucyl-*N,O*-dimethylthreonine, and *N*-methyl-3-phenyllactylamide were identified by GC/MS analysis of the partial acid hydrolyzate after derivatization by successive treatment with methyl trifluoroacetate and B_2D_6, followed by trimethylsilylation.[10] The formation of *N*-methyl-3-phenyllactylamide together with the acid lability of *N*-methyldehydroalanine implied that a 3-phenyllactyl-*N*-methyldehydroalanine moiety was present in the FR900359 molecule. This is in agreement with the structure of **1** discussed earlier.



		a	b	c	d	e+2H	f	g+2H	u+2H	v+2H	w+2H	x	y+2H	z+2H	$(M+H)^+$
9	: R=H	186	287	315	444	462	615	633	609	480	351	333	180	162	794
10	: R=Ac	-	-	399	528	546	-	-	651	480	-	-	-	-	878

Figure 4. Structures of **9** and **10** with an indication of the fragment ions observed in their FAB mass spectra.

Methanolysis of FR900359 with 3 N methanolic hydrochloric acid gave one major segment (**9**). The FAB mass spectrum of **9** exhibited a prominent $(M + H)^+$ ion at m/z 794 and informative sequence ions (Figure 4). Complete hydrolysis of **9** followed by GC/MS analysis indicated that it contains β-hydroxyleucine, 3-phenyllactic acid, and *N*-methylthreonine, but neither alanine nor *N*-methylalanine.

Treatment of **9** with diazomethane resulted in the recovery of the starting material, indicating the absence of a free carboxyl group. On the other hand, acetylation of **9** showed that it had two functional groups susceptible to acetylation, which were located on the first and second units from the N-terminus.

The structure of **9** was deduced from the data discussed above and the comparison of its mass spectrum with that of the acetylated product (**10**), as shown in Figure 4.

Partial hydrolysis of FR900359 in constant boiling hydrochloric acid at 110 °C for 30 min gave ten segments (Table II). The product (**11**) of highest molecular weight (1019) was found to have the sequence shown in Figure 5, on the basis of its FAB mass spectrum and the simpler spectra of the smaller components.

Since the molecular weight of FR900359 is 1001, it must be a cyclic depsipeptide on the basis of all the data presented above. The C-terminal methylamide and the N-

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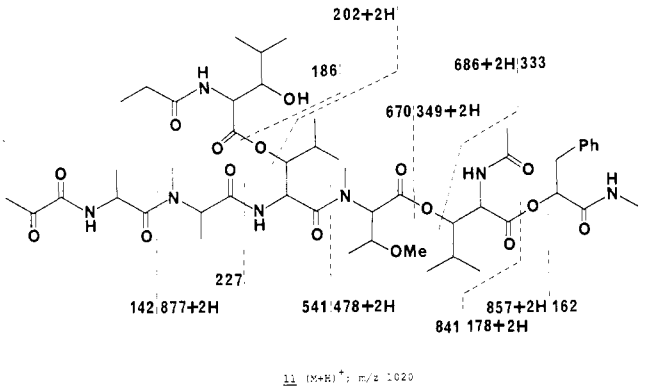
Table II. Tentative Structures of the Products from the Partial Acid Hydrolysis of FR900359[a]

Segment ($M+H^+$, m/z)	Tentative structure and FAB mass fragmentations
12 (319)	(N,O-diMe-Thr)-O-(N-Ac-β-OH-Leu)
13 (535)	(β-OH-Leu)-(N,O-diMe-Thr)-O-(β-OH-Leu) C+2H(406), B(388)
14 (577)	(β-OH-Leu)-(N,O-diMe-Thr)-O-(N-Ac-β-OH-Leu) C+2H(406), B(388)
15 (696)	(β-OH-Leu)-(N,O-diMe-Thr)-O-(β-OH-Leu)-O-(N-Me-3-Ph-Lactylamide) C+2H(406), B(388), C+2H(537), B(517), Y+2H(309)
16 (752)	(N-propionyl-β-OH-Leu)-(β-OH-Leu)-(N,O-diMe-Thr)-O-(β-OH-Leu)-O-(N-Me-3-Ph-Lactylamide) B(186), z(420), C+2H(462), B(444), B(573), C+2H(333), C+2H(591)
17 (738)	(β-OH-Leu)-(N,O-diMe-Thr)-O-(N-Ac-β-OH-Leu)-O-(N-Me-3-Ph-Lactylamide) C+2H(406), B(388), z(333), C+2H(577), B(559)
9 (794)	See Fig. 4
18 (894)	Ala-(N-Me-Ala)-(β-OH-Leu)-(N,O-diMe-Thr)-O-(N-Ac-β-OH-Leu)-O-(N-Me-3-Ph-Lactylamide) (β-OH-Leu), Y+2H(480), z(333), B(415), C+2H(562), C+2H(733)
19 (964)	(Pyruvinyl-Ala)-(N-Me-Ala)-(β-OH-Leu)-(N,O-diMe-Thr)-O-(N-Ac-β-OH-Leu)-O-(N-Me-3-Ph-Lactylamide) (β-OH-Leu), B(227), z(333), B(485), Y+2H(480), C+2H(516), C+2H(632), C+2H(803)
11 (1020)	See Fig. 5

[a] Abbreviations: Ala; alanine, *N*-Me-Ala; *N*-methylalanine, β-OH-Leu; β-hydroxyleucine, *N*-Ac-β-OH-Leu; *N*-acetyl-β-hydroxyleucine, *N*,*O*-DiMe-Thr; *N*,*O*-dimethylthreonine, *N*-Me-3-Ph-Lactylamide; *N*-methyl-3-phenyllactylamide.



11 $(M+H)^+$; m/z 1020

Figure 5. Structure of **11** with an indication of the fragment ions observed in its FAB mass spectrum.

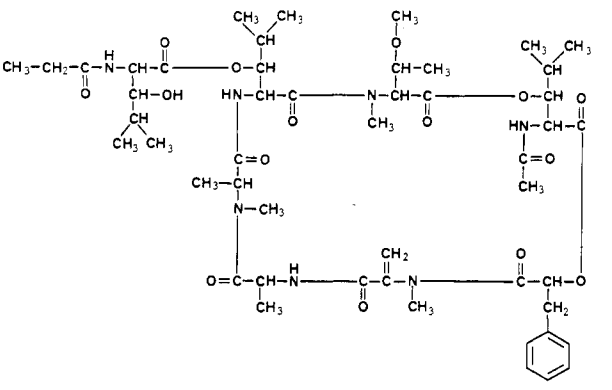


Figure 6. Structure of FR900359.

terminal pyruvic acid residues of **11** must have been generated by the hydrolytic cleavage of the *N*-methyldehydroalanine residue of the original molecule. Therefore, the cyclic structure shown in Figure 6 is proposed for FR900359. It is in complete agreement with the subunits **1** and **9** and all the partial acid hydrolysis products listed in Table II, as well as the ^1H and ^{13}C NMR data shown in Table I.

The FR900359 molecule has a number of interesting features: firstly, the novel amino acid *N*,*O*-dimethylthreonine; secondly, the macrocyclic 22-member ring [3-acetamido-22-benzyl-10-[1-[(3-hydroxy-4-methyl-2-propionamidopentanoyl)oxy]-2-methylpropyl]-4-isopropyl-7-(1-methoxyethyl)-19-methylene-8,13,14,16,20-pentamethyl-1,5-dioxa-8,11,14,17,20-pentaazacyclodocosane-2,6,9,12,18,21-heptone]; and lastly, the susceptibility of the *N*-methyldehydroalanine residue to nucleophilic attack as shown in the ammonolysis, which may be involved in the biological activity of FR900359.

In order to further confirm the structure of FR900359 and to determine the absolute configurations of the constituents, an X-ray crystallographic study of this molecule is in progress. Data obtained so far for all the non-hydrogen atoms of the molecule indicate that this structure is correct (*R* value is 9.8%). The complete results will be reported elsewhere.[7]

Experimental Section

A Varian MAT 731 mass spectrometer equipped with a FAB gun purchased from Ion Tech Ltd. was employed with xenon as the ionizing gas for FAB mass spectral measurements. Glycerol,

glycerol–water (1:1), or glycerol containing 0.05% acetic acid was used as a matrix. HREI mass measurements were performed on a JEOL JMS-01SG-2 mass spectrometer operated at 70 eV. A Varian MAT 212 GC mass spectrometer operated in the EI mode at 70 eV and equipped with a capillary column (J & W: DB-5, film thickness 0.25 μm, 0.32 mm × 15 m) was used for GC/MS analyses, in which the column temperature was raised from 45 to 300 °C at a rate of 10 °C/min. A JEOL JGC-20K gas chromatograph was used for GC analyses. IR spectra were recorded on a Shimadzu IR-420 spectrophotometer. NMR spectra were determined at 100 MHz for proton and 25.1 MHz for carbon-13 spectra on a JEOL PFT-100 spectrometer, using deuteriochloroform as a solvent and tetramethylsilane as an internal standard.

Amino acid analyses were carried out on a Beckmann automatic amino acid analyzer. High performance liquid chromatography (HPLC) was performed on a Waters system consisting of two 510 pumps, a 680 automated gradient controller, a 480 LC spectrophotometer operated at 214 nm, and a U6K injector. A linear gradient solvent delivery system from 0% to 70% acetonitrile (containing 0.035% trifluoroacetic acid) in water (containing 0.05% trifluoroacetic acid) in 30 min was used with a Waters μ-Bondapak C_{18} column (3.9 mm × 30 cm) at a flow rate of 1.5 mL/min.

Isolation of FR900359.[8] FR900359 was isolated from *Ardisia crenata sims* and purified at the Tokyo research laboratories of Fujisawa Pharmaceutical Co., Ltd. A total of 160 kg of the whole plants was homogenized in 540 L of methanol–water (8:2) and allowed to stand for 4 days. The homogenized materials were filtered, and the filtrate was reduced to about 40 L at reduced pressure and extracted twice with 40 L of ethyl acetate. The combined ethyl acetate layer was evaporated to dryness at reduced pressure to give 420 g of crude extract. The crude extract was dissolved in 4 L of diethyl ether. The diethyl ether layer was washed successively with 8 L of 0.1 N sodium hydroxide and water saturated with sodium chloride and then dried over anhydrous sodium sulfate. After filtration, the diethyl ether solution was evaporated to dryness at reduced pressure to give 121 g of material.

In the following three chromatographic procedures, each fraction was tested for activity in decreasing the blood pressure of anesthetized normotensive rats, and active fractions were collected. Half of the material was applied to a silica gel column (Merck, Kieselgel 60, 0.063–0.200 mm, 7.5 cm × 50 cm) and developed with benzene–ethanol (10:1). After 2 L of eluent had passed through the column, the active fraction (2–3 L) was collected and evaporated to dryness at reduced pressure. The second half of the material was purified by the same procedure. The combined fractions (31 g after evaporation of the solvent) were further chromatographed on a silicic acid column (Mallinckrodt, CC-7 Special, 5.5 cm × 51 cm) using *n*-hexane–acetone (2:1) as eluent. The active fraction (2–4 L) was collected and evaporated to dryness at reduced pressure to give 2.1 g of material. The material was purified by Sephadex LH 20 (3.5 cm × 46 cm) column chromatography using methanol as developing solvent. After 180 g of eluent had passed through the column, the active fraction (180–200 g) was collected and evaporated to dryness at reduced pressure to give 1.65 g of crude product which was crystallized from *n*-hexane to give 1.1 g of FR900359. Anal. Calcd for $C_{49}H_{75}N_7O_{15}$: C, 58.73; H, 7.54; N, 9.79. Found: C, 58.66; H, 7.72; N, 9.83. IR (KBr): 3300 (NH and OH), 1750 (ester C=O), 1665 and 1635 (amide C=O) cm^{-1}. HREIMS: *m/z* 1001.5402 (calcd for $C_{49}H_{75}N_7O_{15}$ 1001.5321). FABMS: *m/z* 1002 $(M + H)^+$. See Table I for ^1H and ^{13}C NMR spectral data.

Hydrolysis. A sample of 0.2 mg of FR900359 in 0.5 mL of constant boiling HCl (Pierce, sequanal grade) was freeze-degassed, evacuated, sealed, and heated at 110 °C for 20 h. The mixture was evaporated to dryness at reduced pressure. An aliquot of the hydrolyzate was dissolved in a small amount of 0.02 N HCl and a small portion of the solution was subjected to automatic amino acid analysis which indicated the presence of alanine, *N*-methylalanine, β-hydroxyleucine, and methylamine at a 1.0:1.0:2.2:1.2 molar ratio. The remainder of the hydrolyzate was sonicated in 0.5 mL of 10% HCl in anhydrous butanol (Regis) at room temperature for 15 min and then heated at 90 °C for 30 min in a sealed vial. The mixture was evaporated to dryness at reduced pressure, treated with 0.5 mL of 25% trifluoroacetic anhydride in methylene chloride at room temperature for 1 h, and evaporated to dryness under nitrogen. The residue was dissolved in 200 μL of methylene chloride. An aliquot of the solution was analyzed by GC/MS which indicated the presence of the derivatives of alanine, *N*-methylalanine, β-hydroxyleucine, and 3-phenyllactic acid. The identification of these compounds was confirmed by comparison with commercially available authentic samples.

The solution obtained from the complete hydrolysis of 0.2 mg of FR900359 was neutralized with 6 N sodium hydroxide and adjusted to pH 10 with 0.1 N sodium hydroxide. The solution was evaporated to dryness at reduced pressure and dissolved in a small amount of 0.1 N HCl, and an aliquot of the solution was analyzed by GC on a glass column (2 mm × 2 m) packed with 10% PEG 6000 on a 60–80-mesh Shimalite (Shimadzu); column temperature, 135 °C. Acetic acid and propionic acid were identified by direct comparison with authentic samples.

The solution obtained from the complete hydrolysis of 0.5 mg of FR900359 was neutralized with 6 N sodium hydroxide and acidified with 100 μL of 6 N HCl. To the solution was added 2 mL of 0.35% 1,2-phenylenediamine dihydrochloride (FLUKA) in 0.6 N HCl, and the mixture was allowed to stand for 5 h at room temperature. The solution was extracted four times with 2 mL of ethyl acetate (Pierce, sequanal grade). The combined ethyl acetate layer was reduced to 1 mL under nitrogen and rinsed three times with 1 mL of 0.6 N HCl, and the solution was evaporated to dryness under nitrogen to give the quinoxalinol derivatives of α-keto acids. To the residue dissolved in 0.5 mL of pyridine was added 0.5 mL of *N,O*-bis(trimethylsilyl)trifluoroacetamide, the solution was heated at 60 °C for 30 min, and excess reagents were removed under nitrogen. The trimethylsilylated samples were dissolved in 200 μL of chloroform and an aliquot of the solution was analyzed by GC/MS. For comparison, a mixture of 1 μmol each of pyruvic acid, 2-oxobutyric acid (Aldrich), and 4-methyl-2-oxopentanoic acid (Aldrich) in 1 mL of 1 N HCl was treated with 2 mL of 0.35% 1,2-phenylenediamine dihydrochloride in 0.6 N HCl to produce authentic quinoxalinol derivatives. The mixture was treated in the same manner as described above for comparison.

Hydrolytic Decomposition of β-Hydroxyleucine. A sample of authentic β-hydroxyleucine was hydrolyzed in constant boiling HCl at 110 °C for 20 h in an evacuated and sealed tube and converted to a trimethylsilylated quinoxalinol derivative by treatment with 1,2-phenylenediamine followed by trimethylsilylation in the same manner as described above. GC/MS analysis of the resulting material showed the presence of 4-methyl-2-oxopentanoic acid in the hydrolyzate.

Reduction of *N*-Methyldehydroalanine. A portion of FR900359 was reduced with sodium borodeuteride, essentially as described by Liesch et al.[9] To 0.2 mg of FR900359 in 100 μL of tetrahydrofuran–ethanol (9:1) was added 0.2 mg of sodium borodeuteride (Aldrich), and the solution was allowed to stand for 24 h at room temperature. Subsequently, the solution was acidified by addition of 200 μL of 50% aqueous acetic acid and the solution was allowed to stand for 18 h at room temperature. The sample was dried at reduced pressure and hydrolyzed in 1 mL of constant boiling HCl at 110 °C for 20 h. The hydrolyzate was dried at reduced pressure and converted to trifluoroacetyl butyl ester derivatives. The resulting derivatives were dissolved in a small amount of chloroform and an aliquot of the solution was injected into GC/MS. The derivatives of *N*-methylalanine and *N*-methylalanine-*d* co-eluted but were identified on the basis of their mass spectra. Butyl ester of *N*-trifluoroacetyl-*N*-methylalanine: M^+, *m/z* 255; M – OBu, 182; M – COOBu, 154. Butyl ester of *N*-trifluoroacetyl-*N*-methylalanine-*d*: M^+, *m/z* 256; M – OBu, 183; M – COOBu, 155. Intensity ratios of *m/z* 154 to 155, *m/z* 182 to 183, and *m/z* 255 to 256 were approximatley 1.

Segment 1. A solution of 0.2 mg of FR900359 in 0.5 mL of 20% NH_4OH aqueous solution–methanol (1:1) was allowed to stand at room temperature for 48 h in a sealed tube and evaporated to dryness at reduced pressure. The residue was fractionated on HPLC to give one major segment (1): HRFABMS, *m/z* 681.3833 (calcd for $C_{32}H_{53}N_6O_{10}$ 681.3823), 534.2935 (calcd for $C_{26}H_{40}N_5O_7$ 534.2928); see Figure 3 for FAB mass spectral data.

A sample of 1 was hydrolyzed in constant boiling HCl. An aliquot of the hydrolyzate was subjected to automatic amino acid

analysis to give alanine, *N*-methylalanine, β-hydroxyleucine, methylamine, and an unidentified amino compound. The remainder of the hydrolyzate was converted to trifluoroacetyl or pentafluoropropionyl butyl ester derivatives according to the method described above and dissolved in a small amount of chloroform, and a small portion of the solution was injected into the GC/MS, showing the derivatives of alanine, *N*-methylalanine, β-hydroxyleucine, 3-phenyllactic acid, and two new compounds, 3-amino-2-(methylamino)propionic acid and *N*-methylthreonine. The EI fragments of the butyl ester of trifluoroacetylated (**2**) and of pentafluoropropionylated (**3**) 3-amino-2-(methylamino)-propionic acid are shown in Figure 1; those of the butyl ester of trifluoroacetylated (**4**) and of pentafluoropropionylated (**5**) *N*-methylthreonine are depicted in Figure 2.

Ammonolysis of FR900359 with 20% $^{15}NH_4OH$ aqueous solution (Cambridge Isotope Laboratories)–methanol (1:1) gave **1**(^{15}N) in the same manner: FAB mass spectrum, see Figure 2. A sample of **1**(^{15}N) was hydrolyzed and converted to trifluoroacetyl butyl ester derivatives and analyzed in the same manner as described above for **1** to give the derivatives of alanine, *N*-methylalanine, β-hydroxyleucine, 3-phenyllactic acid, 3-(^{15}N)-amino-2-(methylamino)propionic acid, and *N*-methylthreonine. The EI fragments of the butyl ester of trifluoroacetylated 3-(^{15}N)amino-2-(methylamino)propionic acid (**6**) are shown in Figure 1.

A solution obtained from the complete hydrolysis of a sample of **1** in 0.5 mL of constant boiling HCl was treated with 1,2-phenylenediamine and trimethylsilylated. Analysis by GC/MS in the same manner as described above indicated the presence of 2-oxobutyric acid and 4-methyl-2-oxopentanoic acid.

Methyl Ester of Acetylated 1 (7) and Methyl Ester of 2,2,2-Trideuterioacetylated 1 (8). An aliquot of **1** was esterified in 0.5 mL of 3 N methanolic HCl at room temperature for 30 min with sonication and evaporated to dryness at reduced pressure to give the methyl ester of **1**. An aliquot of the methyl ester of **1** was acetyalted in 0.5 mL of acetic anhydride–pyridine (1:1) and, alternatively, with 2,2,2-trideuterioacetic anhydride (Merck)–pyridine (1:1) at 60 °C for 30 min, and evaporated to dryness to give **7** or **8**, respectively. The FABMS fragments are listed in Figure 3.

Trifluoroethyl *O*-Trimethylsilyl Polyamino Alcohols. An evacuated and sealed tube containing 0.2 mg of FR900359 in 0.5 mL of constant boiling HCl was heated at 110 °C for 30 min. The mixture was evaporated to dryness at reduced pressure and converted to trifluoroethyl *O*-trimethylsilyl polyamino alcohol derivatives according to the method reported by Carr et al.[10] The derivatives were dissolved in 200 μL of methylene chloride, and an aliquot of the solution was analyzed by GC/MS to give eight compounds. EI mass spectrum of derivative of *N*-acetyl-β-hydroxyleucine, *m/z* (relative intensity): 294 (5), M – Me; 266 (6), M – $CHMe_2$; 204 (76), M – CD_2OSiMe_3; 164 (100), M – $HCOSiMe_3CHMe_2$; 148 (24); ?; 114 (5), 204 – $HOSiMe_3$; 102; ?; 73 (44), $SiMe_3$. *N*-Propionyl-β-hydroxyleucine: 308 (1), M – Me; 280 (1), M – $CHMe_2$; 218 (45), M – CD_2OSiMe_3; 178 (100), M – $CHOSiMe_3CHMe_2$; 162 (21), ?; 128 (10), 218 – $HOSiMe_3$; 118 (9), ?; 73 (68), $SiMe_3$. *N*-Methyl-3-phenyllactylamide: 224 (8), M – Me; 193 (34), M – CD_2NHMe; 192 (17), ?; 149 (45), M – $HOSiMe_3$; 148 (12), M – 91; 91 (11), C_7H_7; 73 (100), $SiMe_3$. Alanyl-*N*-methylalanyl-β-hydroxyleucyl-*N*,*O*-dimethylthreonine: 411 (1), M – 201 (Z_2); 406 (1), M – 206 (A_3 + 16); 390 (29), M – 222 (A_3); 261 (9), 406 – $CHOSiMe_3CHMe_2$; 222 (62), $CD_2NMeCH(CHOMeMe)CD_2OSiMe_3$ (Z_1); 217 (22), A_2 + 16; 201 (24), $CF_3CD_2NHCHMeCD_2NMeCHMe$ (A_2); 173 (16), $CF_3CD_2NHCHMeCD_2NMe$; 163 (13), 222 – CHOMeMe; 144 (15), $CF_3CD_2NHCHMeCD_2$ (A_1 + 16); 105, CD_2OSiMe_3; 73 (100), $SiMe_3$. The remaining four derivatives were found to be the derivatives of β-hydroxyleucine, 3-phenyllactic acid, alanyl-*N*-methylalanine, and β-hydroxyleucyl-*N*,*O*-dimethylthreonine, but their mass spectra are not shown. The notation A_n and Z_n is that of Nau and Biemann.[11]

Segment 9. To 0.2 mg of FR900359 was added 0.5 mL of 3 N methanolic HCl. The solution was heated at 75 °C for 50 min in a sealed tube, evaporated to dryness at reduced pressure, and then subjected to HPLC fractionation to give one major segment (**9**). See Figure 4 for FAB mass spectral data.

An aliquot of **9** was hydrolyzed in constant boiling HCl, converted to trifluoroacetyl butyl esters according to the same method described above, and analyzed by GC/MS to give the derivatives of β-hydroxyleucine, 3-phenyllactic acid, and *N*-methylthreonine.

The segment (**9**) was esterified in 0.5 mL of etheral diazomethane and evaporated to dryness under a stream of nitrogen to give a residue, whose FAB mass spectrum was identical with that of **9**. Another aliquot of **9** was acetylated in 0.5 mL of acetic anhydride–pyridine (1:1) at 60 °C for 30 min and evaporated to dryness to give acetylated **9** (**10**). For FAB mass spectral data, see Figure 4.

Segment 11. To 0.2 mg of FR900359 was added 0.5 mL of constant boiling HCl, and the solution was heated to 110 °C for 30 min in a evacuated and sealed tube. The partial hydrolyzate was evaporated to dryness at reduced pressure and fractionated by HPLC to give a segment (**11**) having a molecular weight of 1019 based on FABMS and nine segments , **9** and **12** – **19**. FAB mass spectral data of **11** are summarized in Figure 5 and those of segments **12**–**19** in Table II.

Acknowledgment. We thank Markus Zollinger at MIT for useful discussions. This work would not have been possible without the advice of Stephen A. Martin and Catherine E. Costello (MIT Mass Spectrometry Facility) who also provided some of the FAB spectra. We also thank Dr. Hagime Ishijima (JEOL Ltd.) for HREI mass spectral measurements. The work at MIT was supported by a grant from the U.S. National Institutes of Health (RR00317).

Supplementary Material Available: Mass spectra of **1**, **2**, **4**, **9**, **11**, and the trifluoroethyl *O*-trimethylsilyl polyamino alcohol derivatives of *N*-methyl-3-phenyllactylamide and alanyl-*N*-methylalanyl-β-hydroxyleucyl-*N*,*O*-dimethylthreonine; tabulated mass spectral data of **3**, **5**, and **6** (8 pages). Ordering information is given on any current masthead page.

(11) Nau, H.; Biemann, K. *Anal. Biochem.* **1976**, *73*, 154.