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Synthesis and Conformational Analysis of Cyclo-TRI[L-Valyl-D-Hexahydromandelyl]

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The synthesis of the cyclo-hexadepsipeptide [L-valyl-D-hexahydromandelyl]$_3$ is described. Examination of this macrocyclic compound by 220-MHz nuclear magnetic resonance spectroscopy shows that symmetrical conformations are stabilized in strongly polar solvents (trifluoroacetic acid, acetonitrile), whereas asymmetric conformations are preferred in nonpolar or slightly polar media such as carbon tetrachloride, chloroform, cyclohexane, and benzene.

From analysis of the temperature dependence of the chemical shift and of the coupling constants, together with conformational energy calculations, a model is proposed for the preferred conformation of this molecule in nonpolar solvents.

The relationships between ion-complexing ability, biological activity, and the ability to transport ions through membranes for cyclodepsipeptides and cyclopeptides have been thoroughly investigated in recent years (*1*).

Cyclodepsipeptide antibiotics such as valinomycin (*2*) and enniatin B (*3*) have been shown to complex with alkali metal cations and to affect the permeability of biological and artificial lipid membranes toward certain monovalent cations (*4*). Under nonaqueous conditions, valinomycin (*5*) demonstrates a remarkable selectivity for potassium ions as compared to sodium ions. These properties are undoubtedly related to the conformation of the cyclodepsipeptides and the membranes involved. Recently, investigators have shed some light on the structure and conformation of cyclic peptides and cyclic depsipeptides by means of proton magnetic resonance and semiempirical calculations. In addition to the cyclic depsipeptides noted above, structures for gramicidin-S (*6–9*), antamanide (*10–12*) and the cyclolinopeptide A (*13–15*) have been investigated. The present work was undertaken to study the conformational properties in solution of the cyclohexadepsipeptide (L-valyl-D-hexahydromandelyl)$_3$ (XII), an analog of enniatin B. This compound contains an 18-membered ring and β-branched side chains. These features have been shown to be related to the membrane permeability and antimicrobial activity of enniatin B (*16*). It has been suggested (*4*) that the greater stability of the valinomycin–K^+ complex, as compared to the enniatin–K^+ complex, may be due to a more effective shielding from solvent of the bound cation and the amide

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and ester groups by its 36-membered ring and hydrophobic side chains. The presence of large and relatively rigid hydrophobic side chains in the newly synthesized 18-membered cyclodepsipeptide should produce an increase in the formation constant of alkali metal complexes and in the ion selectivity—with respect to enniatin B—by affecting the solvation energy of the complex ion.

The synthesis of XII is outlined in Fig. 1 and details of the synthesis are included in the experimental section. The compound D-hexahydromandelic acid was prepared by hydrogenation of D-mandelic acid with either 5% rhodium on alumina (*17*) or platinum oxide as catalyst. In order to prepare *tert*-butyl-D-hexahydromandelate, it was first

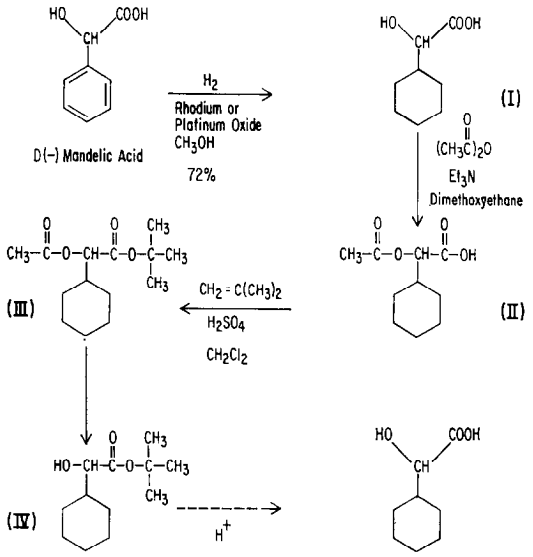


FIG. 1a

necessary to protect the α-hydroxyl group of D-hexahydromandelic acid. Attempts to prepare *O*-(benzyloxycarbonyl)-D-hexahydromandelic acid by two different methods (*18*, *19*) gave products in low yields. However, α-acetoxy-D-hexahydromandelic acid was conveniently prepared in better than 95% yield.

This compound was then used to synthesize *tert*-butyl-α-acetoxy-D-hexahydromandelate (*20*) from which *tert*-butyl-D-hexahydromandelate was obtained in 78% overall yield by mild base-catalyzed hydrolysis. Acid-catalyzed hydrolysis of the ester gave optically pure hexahydromandelic acid indicating the absence of racemization during the previous steps.

Attempts to prepare *N*-benzyloxycarbonyl-L-valyl-*tert*-butyl-D-hexahydromandelate (V) by activation of *N*-benzyloxycarbonyl-L-valine (*21*) with benzene sulfonyl chloride and then condensation with *tert*-butyl-D-hexahydromandelate in pyridine (*18*) gave a mixture of products and low yields. The ester (V) was then conveniently prepared by activation with *N*,*N*′-carbonyldiimidazole (*22*). The compound L-valyl-*tert*-butyl-D-hexahydromandelate (VII) was prepared by hydrogenolysis of V, whereas treatment of

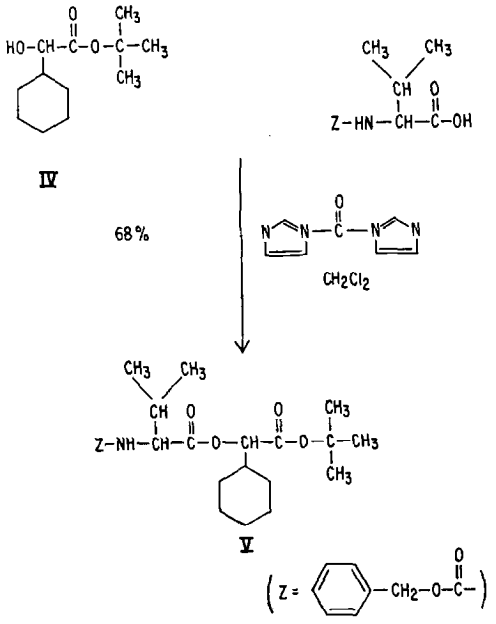

IV
68%
CH2Cl2
V
(Z = CH2–O–C–)

FIG. 1b

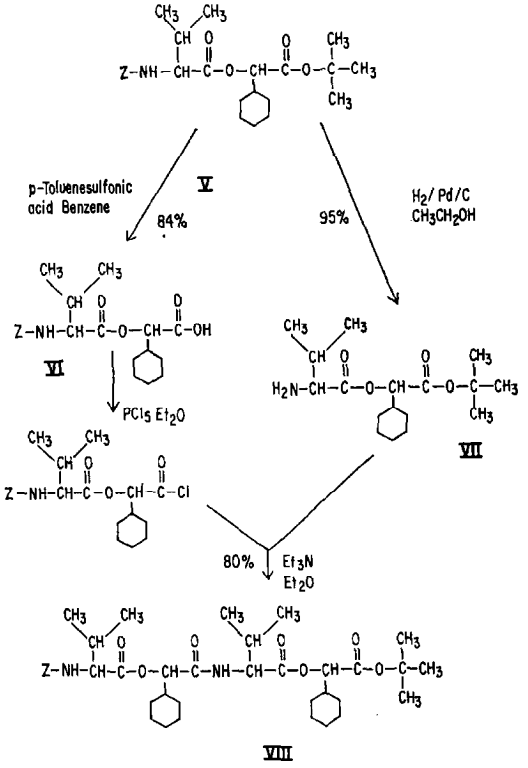
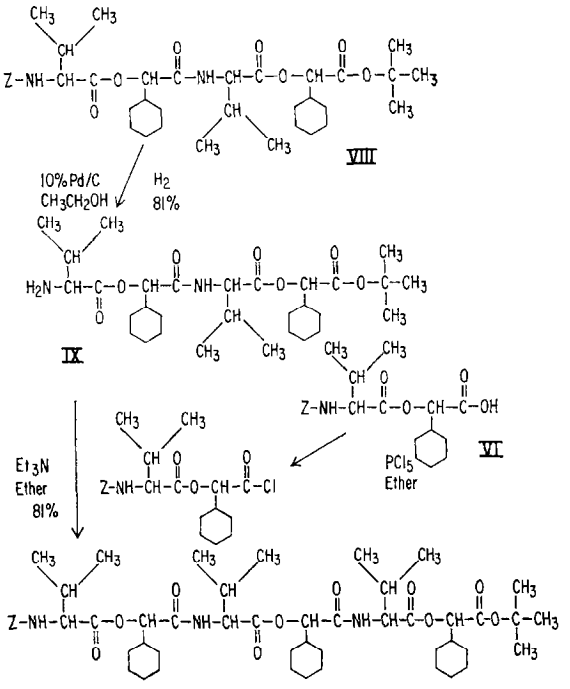
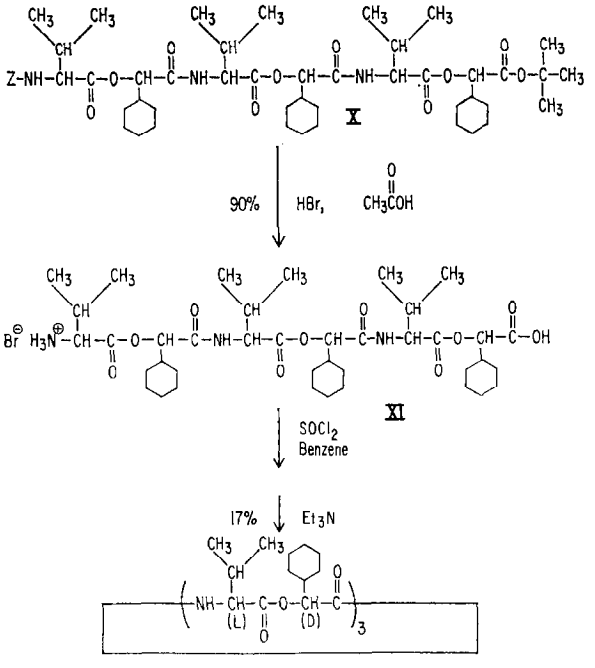

V
p-Toluenesulfonic acid Benzene
84%
95%
H2/Pd/C
CH3CH2OH
VI
PCl5 Et2O
VII
80%
Et3N
Et2O
VIII

FIG. 1c



VIII IX VI X

FIG. 1d



X XI XII

FIG. 1e

FIG. 1. (a–e). Schematic representation of the synthesis of the cyclohexadepsipeptide.

V with p-toluenesulfonic acid yielded *N*-benzyloxycarbonyl-L-valyl-D-hexahydromandelic acid (VI). Peptide coupling between (VI) and (VII) using the acid chloride method produced the tetradepsipeptide (VIII) from which L-valyl-D-hexahydromandelyl-L-valyl-*tert*-butyl-D-hexahydromandelate (IX) was obtained after hydrogenolysis. *N*-Benzyloxycarbonyl-L-valyl-D-hexahydromandelic acid (VI) was then coupled with IX by the acid chloride method to give the blocked hexadepsipeptide (X). The hexadepsipeptide hydrobromide (XI) was obtained by removing the protecting groups from (X) by treatment with acetic acid saturated with hydrogen bromide. High dilution cyclization of (XI) by the acid chloride method gave cyclo-(L-valyl-D-hexahydromandelyl)$_3$ (XII) in ca. 17% yield.

EXPERIMENTAL SECTION

1. *Synthesis*

D(−)*Hexahydromandelic acid* (I)

D(−)Mandelic acid (30.4 g, 0.2 mole), dissolved in 160 ml of absolute methanol and 2.0 ml of glacial acetic acid, was hydrogenated in a Parr shaker apparatus, using 6.0 g of 5% rhodium on alumina as catalyst, at a pressure of 50 psi for 3 hr at room temperature. The mixture was filtered and the solvent removed under vacuum, yielding 28 g of I.

After recrystallization of the crude material from benzene–acetone (4:1 v/v), 22.8 g colorless crystals were obtained (yield 72%). mp = 129–130°C (lit.[17]: 129°C); $[\alpha]_D^{27}$ = −22.6 (C = 1 in glacial acetic acid) [lit.[22]: $[\alpha]_D^{20}$ = −25.5 (C = 1 in glacial acetic acid)]. ir (KBr): 3450 cm^{-1} (νOH); 1715 cm^{-1} (νC = 0).

Anal. Calcd for $C_8H_{14}O_3$: C, 60.75; H, 8.86. Found: C, 60.81; H, 8.82.

tert-Butyl-D-hexahydromandelate

(a) *α-Acetoxy-D-hexahydromandelic acid* (*II*). To a cooled solution of 14.2 g (0.09 mole) of D-hexahydromandelic acid in 90 ml anhydrous dimethoxyethane and 27.9 ml (0.198 mole) triethylamine, 9.3 ml (0.099 mole) of acetic anhydride was added over a period of 10 min. The reaction mixture was kept in the cold 30 min and then stirred at room temperature for 24 hr. The solvent was removed under vacuum and the oily residue dissolved in ether. This solution was washed with water, 4 *M* hydrochloric acid and extracted with saturated potassium bicarbonate. The aqueous phase was acidified with 6 *M* hydrochloric acid and extracted with ether. After washing with saturated aqueous potassium chloride and drying over magnesium sulfate, the ether was removed under reduced pressure to give a chromatographically homogeneous oil. Yield 18.0 g (95%). $[\alpha]_D^{26}$ = 6.25 (C = 1.2 in ethanol). ir (KBr): 1760 cm^{-1} (νC = O of the ester); 1720 cm^{-1} (νC=O of the acid). This compound was used with no further purification.

(b) *tert-Butyl-α-acetoxy-D-hexahydromandelate* (*III*). α-Acetoxy-D-hexahydromandelic acid (3.0 g, 0.015 mole) was dissolved in 30 ml dichloromethane and cooled in an acetone–dry ice bath. To the cold solution, 15 ml (0.15 mole) of isobutylene and 0.5 ml of concentrated sulfuric acid were added. After shaking, the solution was allowed to stand 94 hr at room temperature. The reaction mixture was poured into a beaker, diluted with 100 ml dichloromethane and stirred with 25 ml of 10% sodium carbonate. The organic phase was then washed with water, 10% sodium carbonate, and saturated

aqueous sodium chloride. After drying over magnesium sulfate, the solvent was removed, yielding 4.0 g of a clear oil. A chromatographically homogeneous sample (3.5 g, >90%) was obtained by elution of the product with benzene in a silica gel column. $[\alpha]_D^{25} = 29.1$ (C = 1.1 in 95% ethanol). ir (thin film): 1740 cm^{-1} (νC=O). This material was used with no further purification.

(c) *tert-Butyl-*D*-hexahydromandelate* (IV). To a solution of 3.8 g (0.015 mole) of *tert*-butyl-α-acetoxy-D-hexahydromandelate in 6.5 ml methanol–water mixture (5:1.5 v/v), 8 ml 2 *M* sodium hydroxide were added dropwise over a period of 50 min, while cooling in an ice bath. The solution was allowed to warm to room temperature and stirred for 4 additional hr. The reaction mixture was then diluted with water and extracted three times with 10 ml of ether. The ethereal phase was washed with water and saturated aqueous sodium chloride, dried over magnesium sulfate, and the solvent removed under reduced pressure. The residue (3.2 g) was eluted with hexane–dichloromethane (2:1 v/v) and dichloromethane–ethyl acetate (49:1 v/v) in a silica gel column.

After removal of the solvent, a colorless liquid was obtained (2.5 g, 78%). $[\alpha]_D^{25} = 0.9$ (C = 1 in ethanol). ir (thin film): 3530 cm^{-1} (νOH); 1720 cm^{-1} (νC=O).

Anal. Calcd for $C_{12}H_{22}O_3$: C, 67.26; H, 10.35. Found: C, 66.96; H, 10.21.

*N-Benzyloxycarbonyl-*L*-valyl-tert-butyl-*D*-hexahydromandelate (V)*

To a stirred solution of *N,N'*-carbonyldiimidazole (10.5 g, 0.065 mole) in 65 ml of anhydrous dichloromethane, a solution of *N*-benzyloxycarbonyl-L-valine (16.3 g, 0.065 mole) in 45 ml of anhydrous dichloromethane was added at 0°C over a period of 30 min, followed by *tert*-butyl-D-hexahydromandelate (10.7 g, 0.050 mole). After an additional hour at 0°C, the ice bath was removed and the reaction mixture stirred at room temperature for 90 hr. Then 10 ml of water was added to destroy the excess of activated acid, and the solvent was removed under reduced pressure. The residue was dissolved in ether and washed with water, 5% citric acid, water, saturated sodium bicarbonate and water. The ethereal fraction was dried over magnesium sulfate and the solvent removed under reduced pressure to give a clear oil (22.0 g) which was crystallized from hexane, yielding 15.0 g (68%) of colorless crystals. mp = 66–67°C; $[\alpha]_D^{25} = +2.6$ (C = 1 in ethanol). ir (KBr): 3320 cm^{-1} (νNH); 1748 cm^{-1}, 1720 cm^{-1}, 1705 cm^{-1} (νC=O).

Anal. Calcd for $C_{25}H_{37}NO_6$: C, 67.09; H, 8.33; N, 3.13. Found: C, 67.30; H, 8.42; N, 3.24.

*N-Benzyloxycarbonyl-*L*-valyl-*D*-hexahydromandelic acid (VI)*

A solution of benzyloxycarbonyl-L-valyl-*tert*-butyl-D-hexahydromandelate (1.0 g, 2.2 mmole) and *p*-toluenesulfonic acid (0.093 g) in 10 ml of benzene was heated to reflux for 90 min. The solvent was removed under reduced pressure and the resulting residue dissolved in 10 ml of ether and extracted with cold 5% sodium bicarbonate solution. This solution was acidified with hydrochloric acid and then extracted with ether. The ethereal fraction was dried over magnesium sulfate and the solvent removed under reduced pressure. The oily residue (0.79 g) crystallized on cooling. Pure VI was obtained by crystallization from hot hexane–benzene (3:2 v/v). Yield: 0.72 g, 84%. mp = 116–117°C; $[\alpha]_D^{25} = -14.9$ (C = 1 in ethanol). ir (KBr): 3310 cm^{-1}, 3260 cm^{-1} (νNH); 1745 cm^{-1}, 1723 cm^{-1}, 1655 cm^{-1} (νC=O).

Anal. Calcd for $C_{21}H_{29}NO_6$: C, 64.43; H, 7.47; N, 3.58. Found: C, 64.43; H, 7.61; N, 3.31.

L-*Valyl-tert-butyl*-D-*hexahydromandelate* (*VII*)

N-Benzyloxycarbonyl-L-valyl-*tert*-butyl-D-hexahydromandelate (8.0 g, 18 mmole) was dissolved in 75 ml absolute ethanol and added to a suspension of 1.6 g prereduced 10% palladium on charcoal in 150 ml of absolute ethanol. The mixture was hydrogenated in a Parr apparatus at a pressure of 8 psi for 3 hr at room temperature. After filtration, the solvent was removed under reduced pressure and the resulting residue dissolved in ether and extracted into 5% citric acid solution. The acidic solution was brought to alkaline pH with saturated sodium bicarbonate and extracted with ether. The ethereal portion was washed with water, dried over magnesium sulfate and the solvent evaporated under reduced pressure to give 5.6 g of a chromatographically homogeneous clear oil, which was used without further purification. $[\alpha]_D^{25} = +40.4$ (C = 1.5 in ethanol). ir (thin film): 3390 cm^{-1}, 3325 cm^{-1} (νNH_2); 1730 cm^{-1} ($\nu C{=}O$).

Anal. Calcd for $C_{17}H_{31}NO_4$: C, 65.14; H, 9.97; N, 4.41. Found: C, 64.95; H, 10.09; N, 4.50.

N-Benzyloxycarbonyl-L-*valyl*-D-*hexahydromandelyl*-L-*valyl-tert-butyl*-D-*hexahydromandelate* (*VIII*)

Phosphorous pentachloride (2.0 g, 10 mmole) was added to a solution of *N*-benzyloxycarbonyl-L-valyl-D-hexahydromandelic acid (2.0 g, 7.8 mmole) in 40 ml of anhydrous ether cooled to 0°C. The solution was stirred 45 min, filtered, and the solvent removed under reduced pressure. The residue was redissolved in anhydrous ether and the solvent removed two consecutive times. The residue was finally dissolved in 40 ml of anhydrous ether and added to a solution of L-valyl-*tert*-butyl-D-hexahydromandelate (2.2 g, 7.8 mmole) and triethylamine (1.6 ml, 11.5 mmole) in 80 ml of anhydrous ether previously cooled to −10°C. The reaction mixture was stirred at 0°C for 5 hr, filtered, and the filtrate washed with water, 5% citric acid, water, 5% sodium bicarbonate, and water. The ethereal fraction was dried over magnesium sulfate and the solvent removed under reduced pressure to give an oil, which crystallized on standing. Recrystallization from hexane gave colorless crystals (4.23 g, 80%). m.p = 97–99°C; $[\alpha]_D^{25} = -4.6$ (C = 1 in ethanol). ir (KBr): 3320–3290 cm^{-1} (νNH), 1745 cm^{-1}, 1733 cm^{-1}, 1690 cm^{-1}, 1640 cm^{-1} ($\nu C{=}O$).

Anal. Calcd for $C_{38}H_{58}N_2O_7$: C, 66.45; H, 8.51; N, 4.08. Found: C, 66.38; H, 8.66; N, 3.93.

L-*Valyl*-D-*hexahydromandelyl*-L-*valyl-tert-butyl*-D-*hexahydromandelate* (*IX*)

N-Benzyloxycarbonyl-L-valyl-D-hexahydromandelyl-L-valyl-*tert*-butyl-D-hexahydromandelate (VIII) (3.0 g, 4.37 mmole) was dissolved in 40 ml of absolute ethanol and added to a mixture of 0.6 g of prereduced 10% palladium on charcoal in 75 ml of absolute ethanol. The mixture was hydrogenated in a Parr shaker apparatus at a pressure of 8 psi for 3 hr at room temperature. After filtration, the solvent was removed under reduced pressure. The residue was dissolved in ether and washed with water, 5% sodium bicarbonate, water, and sodium chloride. The ethereal solution was dried over magnesium sulfate and the solvent removed. The resulting oil was crystallized from hexane,

yielding 1.95 g (81%) of pure IX. mp = 79–80°C; ir (KBr): 3390 cm^{-1}, 3330 cm^{-1} (νNH); 1738 cm^{-1}, 1689 cm^{-1} (νC=O).

Anal. Calcd for $C_{30}H_{52}N_2O_7$: C, 65.19; H, 9.48; N, 5.07. Found: C, 65.34; H, 9.64; N, 4.83.

*N-Benzyloxycarbonyl-*L*-valyl-*D*-hexahydromandelyl-*L*-valyl-*D*-hexahydromandelyl-*L*-valyl-tert-butyl-*D*-hexahydromandelate (X)*

To *N*-benzyloxycarbonyl-L-valyl-D-hexahydromandelic acid (2.5 g, 6.4 mmole) in 40 ml of anhydrous ether, phosphorous pentachloride (1.67 g, 8 mmole) was added at 0°C. Stirring was continued for 45 min. The mixture was then filtered, and the solvent removed under vacuum. The residue was redissolved in 20 ml of anhydrous ether and added to a stirred solution of L-valyl-D-hexahydromandelyl-L-valyl-*tert*-butyl-D-hexahydromandelate (3.53 g, 6.4 mmole) and triethylamine (1.54 ml, 11 mmole) in 25 ml of anhydrous ether at −15°C. Stirring was continued for 4 hr. The solution was then filtered, the filtrate washed with 5% citric acid, water, 5% sodium bicarbonate, water, and saturated sodium chloride solution, and dried over magnesium sulfate. Evaporation of the solvent gave 5.7 g of crude X, which was recrystallized from hexane. mp = 148–149°C. ir (KBr): 3420 cm^{-1}, 3360 cm^{-1} (νNH); 1740 cm^{-1}, 1695 cm^{-1}, 1660 cm^{-1}, 1525 cm^{-1} (νC=O).

Anal. Calcd for $C_{51}H_{79}N_3O_{12}$: C, 66.16; H, 8.54; N, 4.54. Found: C, 66.31; H, 8.60; N, 4.46.

L-Valyl-D-hexahydromandelyl-L-valyl-D-hexahydromandelyl-L-valyl-D-hexahydromandelic acid hydrobromide (XI)

To a solution of *N*-benzyloxycarbonyl-L-valyl-D-hexahydromandelyl-L-valyl-D-hexahydromandelyl-L-valyl-*tert*-butyl-D-hexahydromandelate (X) (5.48 g, 5.7 mmole) in 11 ml of glacial acetic acid, 20 ml of hydrogen bromide-saturated acetic acid was added. After stirring for 6 hr, and removing hydrogen bromide under reduced pressure, the solution was lyophilized. The solid residue was dissolved in ether–chloroform (3:1 v/v), washed with water six times, and dried over magnesium sulfate. The solvent was removed under reduced pressure to give 4.3 g (92%) of chromatographically homogeneous XI. ir (KBr): 3430 cm^{-1} (νNH); 1742 cm^{-1}, 1670 cm^{-1}, 1520 cm^{-1} (νC=O).

Anal. Calcd for $C_{39}H_{66}N_3O_{10}Br$: C, 57.35; H, 8.08; N, 5.14; Br, 9.80. Found: C, 57.50; H, 8.27; N, 5.06; Br, 9.88.

*Cyclo[*L*-valyl-*D*-hexahydromandelyl-]$_3$ (XII)*

The hexadepsipeptide hydrobromide (XI) (0.415 g, 0.5 mmole) was dissolved in 2.0 ml of thionyl chloride and stirred for 30 min at room temperature. The solvent was removed under vacuum, and the resulting acid chloride was dissolved in 1.8 liters of dry benzene. Triethylamine (1.45 ml) in 500 ml of dry benzene was then added dropwise to the acid chloride solution over a period of 2.5 hr. After 18 hr, 0.1 ml triethylamine was added and the solution was stirred for 2 more hr. The solvent was then removed under reduced pressure and the solid residue dissolved in ether. The ethereal portion was filtered, washed with 1 *M* hydrochloric acid, water, saturated sodium bicarbonate, water, saturated sodium chloride, and dried over magnesium sulfate. After removal of the solvent under reduced pressure a solid residue was obtained, which was purified on preparative layers of silica gel, using benzene:ethyl acetate (4:1 v/v) as the developing

solvent. The final product was crystallized from ethanol–water as colorless needles (0.060 g, 17%). mp = 271–273°C. Mass spectrum (70 ev): $M^+ = 718$; ir (KBr): 3400 cm^{-1} (νNH); 1750 cm^{-1}, 1678 cm^{-1}, 1662 cm^{-1}, 1525 cm^{-1} (νC=O).

Anal. Calcd for $C_{39}H_{63}N_3O_9$: C, 65.27; N, 8.79; N, 5.85. Found: C, 65.11; H, 8.81; N, 5.78.

2. *NMR Measurements*

All nmr spectra were recorded on a Varian HR-220 spectrometer equipped with a standard Varian temperature control unit. The nmr spectrum of methanol was used to monitor the temperatures below 18°C while the nmr spectrum of ethylene glycol was employed to monitor the temperatures above 18°C. If not otherwise specified, a standard solute concentration of 2% (g/100 ml) was used. Homonuclear spin decoupling experiments were carried out with the same instrumentation plus an audio oscillator Wavetek model 131A.

RESULTS

The nmr spectra of the cyclohexadepsipeptide (XII) are presented in Figs. 2, 3, and 4. In trifluoroacetic acid (Fig. 2), the spectrum is consistent with a symmetrical conformation: a single peak is observed for all the NH protons of the valine residues ($\Delta\delta = 7.88$

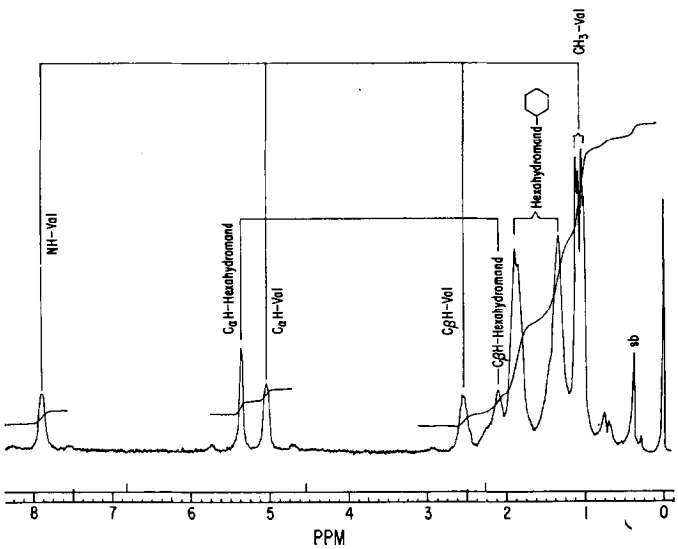


FIG. 2. High resolution 220 MHz nmr spectrum of the cyclohexadepsipeptide in trifluoroacetic acid at −12°C. Internal reference: TMS. Small peaks at 0.73, 2.96, 4.71, 5.76, 7.55, and 8.24 ppm are probably due to a trace contamination of the open-chain hexadepsipeptide.

ppm); single peaks are also exhibited by the α-CH resonances of hexahydromandelic acid ($\Delta\delta = 5.36$ ppm) and valine residues ($\Delta\delta = 5.04$ ppm).

In carbon tetrachloride (Fig. 3) and in chloroform (Fig. 4) separate resonances for each α-CH and NH proton in the valine residue and each α-CH proton of the D-hexahydromandelic acid residue appear, indicating an asymmetric conformation for the macrocyclic compound in these solvents. Similar results, presented in Table 1, also indicate an absence of symmetry in cyclohexane and benzene.

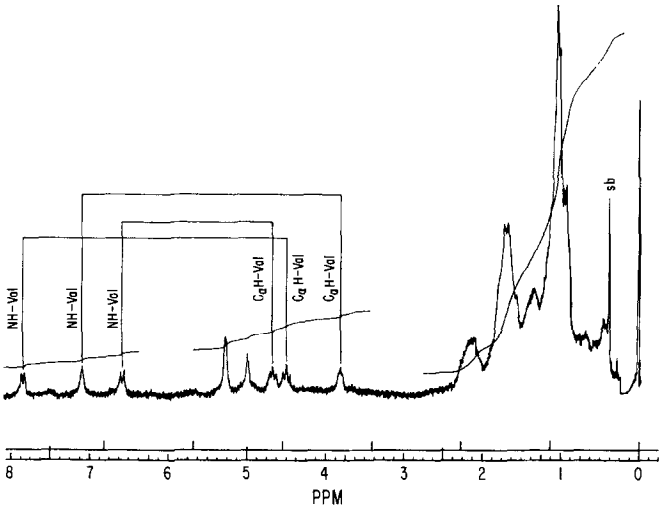


FIG. 3. High resolution 220-MHz nmr spectrum of the cyclohexadepsipeptide in carbon tetrachloride at 18°C. Internal reference: TMS.

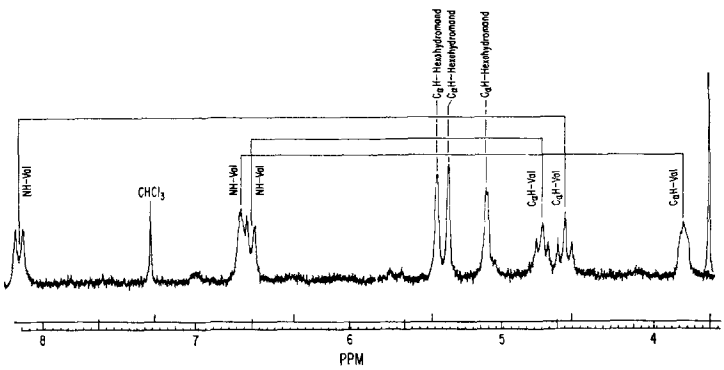


FIG. 4. High resolution 220-MHz nmr spectrum covering the $C_\alpha H$ and NH regions of the cyclohexadepsipeptide in deuterochloroform at −36°C. Internal reference: TMS.

In deuterochloroform, for example, double resonance experiments show that the α-CH protons which appear at 4.56 ppm as a symmetrical triplet ($J = 10 \pm 0.3$ Hz) are coupled to the downfield NH resonance at 8.13 ppm. The α-CH resonance at 3.80 ppm appears as a broad unresolved signal which is coupled to the midfield NH resonance at 6.70 ppm ($J_{N\alpha} \leqslant 2$ Hz). The third α-CH resonance presents an unsymmetrical triplet at 4.72 ppm and is coupled to the NH signal at 6.63 ppm.

TABLE 1

VALYL NH CHEMICAL SHIFTS AND COUPLING CONSTANTS IN DIFFERENT SOLVENTS

Solvent	Chemical shift (ppm from TMS)	$J_{N\alpha}$ (Hz)	Temperature (°C)
Cyclohexane-d_{12}	6.79	9.6 ± 0.3	
	7.62	<2	18
	7.95	9.6 ± 0.3	
	6.54	9.6 ± 0.3	
Carbon tetrachloride	7.08	—	18
	7.82	9.8 ± 0.3	
	7.02	10.0 ± 0.3	
Benzene-d_6	7.71	—	18
	8.47	10.0 ± 0.3	
	6.63	10.0 ± 0.3	
Chloroform-d_1	6.70	—	−36
	8.13	9.5 ± 0.3	
Trifluoroacetic acid	7.88	—	−12
Acetonitrile-d_3	7.27	—	35

A decrease in temperature dependence of the chemical shift of the amide protons has been associated with intramolecular hydrogen bonding in several cyclic peptides and depsipeptides (*9–11*, *23*). Therefore, the chemical shifts of the NH protons of the cyclohexadepsipeptide (XII) were examined as a function of temperature in carbon tetrachloride (Fig. 5), cyclohexane (Fig. 6), and deuterochloroform/tetradeuteromethanol,

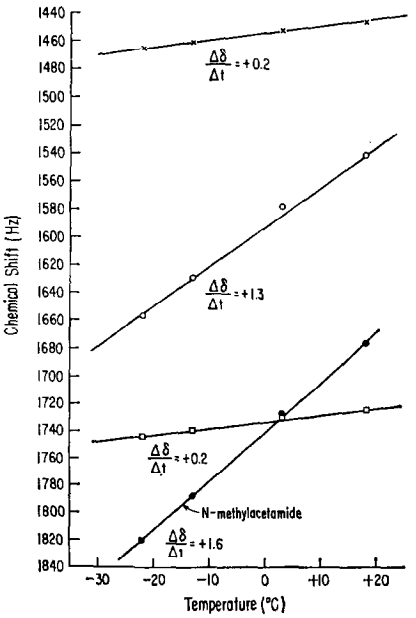


FIG. 5. Temperature dependence of the NH protons chemical shift in carbon tetrachloride.

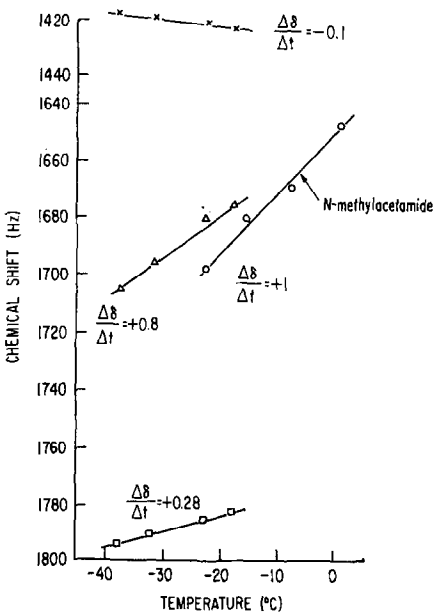


FIG. 6. Temperature dependence of the NH protons' chemical shift in cyclohexane.

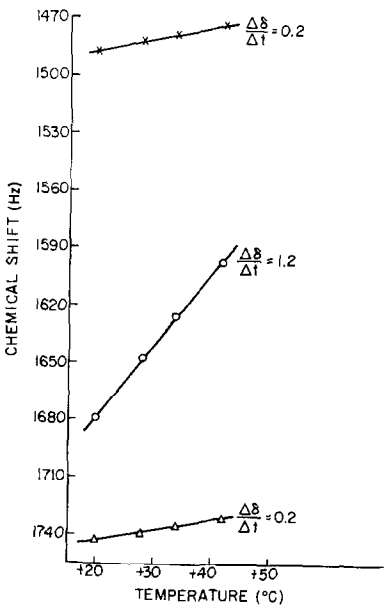


FIG. 7. Temperature dependence of the NH protons' chemical shift in deuterochloroform and tetradeutero-methanol (2:1, v/v) mixture.

2:1 (Fig. 7), using *N*-methylacetamide as a standard. In each solvent the chemical shift of the low field and the high field NH protons showed a small temperature coefficient, compared to *N*-methylacetamide. The chemical shift of the midfield NH proton, however, showed a temperature dependence comparable to *N*-methylacetamide. Furthermore, the chemical shifts of the high- and lowfield NH proton in carbon tetrachloride are insensitive to changes in concentration, shifting downfield only 0.05 ppm when changing from 1.1% to 3.4% concentration (Table 2). The midfield NH proton, however, shifts downfield 0.24 ppm over the same concentration range. These results strongly suggest that the lowfield and upfield NH protons are participating in intramolecular hydrogen bonding while the midfield NH proton is not internally buried and, therefore, capable of interacting with the solvent. It is conceivable that in solvents of low dielectric constant the polar groups would prefer to be buried in the cavity of the molecule and surrounded by the hydrophobic side chains. This is consistent with the fact that nmr spectra of compound XII in nonpolar or slightly polar solvents, such as carbon tetrachloride, cyclohexane, chloroform, and benzene (Table 1) indicate an asymmetric structure with intramolecular hydrogen bonds.

TABLE 2

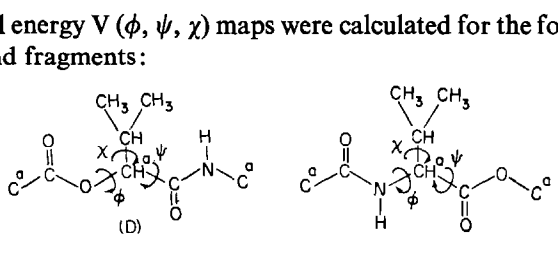
CONCENTRATION-DEPENDENCE OF VALYL NH PROTONS' CHEMICAL SHIFT IN CARBON TETRACHLORIDE

Concentration (% w/v)	Downfield NH Ch. shift (ppm)	Downfield NH $J_{N\alpha}$ (Hz)	Midfield NH Ch. shift (ppm)	Midfield NH $J_{N\alpha}$ (Hz)	Upfield NH Ch. shift (ppm)	Upfield NH $J_{N\alpha}$ (Hz)
1.1	7.80	9.6	6.77	—	6.54	9.5
1.7	7.82	9.8	6.84	—	6.54	9.6
3.4	7.85	10.0	7.1	—	6.58	9.7

In polar solvents, such as trifluoroacetic acid and acetonitrile, the nmr spectra show three broad signals with an intensity ratio of 1:1:1 for the NH and α-CH protons of L-valyl residues and the α-CH proton of D-hexahydromandelyl residues. These results suggest that in solvents of high dielectric constant the polar groups no longer prefer to be buried within the cavity of the molecule, but are sticking outside the ring. The asymmetric structure, held together by intramolecular hydrogen bonds in nonpolar solvents, is then replaced by a symmetrical one, stabilized by interactions of solvent–polar groups.

Semiempirical Calculations:

Conformational energy V (ϕ, ψ, χ) maps were calculated for the following *trans* peptide and ester bond fragments:



(D) (L)

Map A Map B

The potential functions, bond lengths, valence angles, 6–12 nonbonded potential constants, torsional barrier heights, and partial atomic charges employed in these energy calculations were taken from Brant, Miller, and Flory (*24*) and Brant, Tonelli, and Flory (*25*). The constants $C_{N,O}$ and $A_{N,O} - C_{N,O}/r^6_{N,O} + A_{N,O}/r^{12}_{N,O}$ appropriate to the nonbonded van der Waals interactions between the amide nitrogen and the ester oxygen atoms were evaluated in the usual manner (*24*, *25*) and found to be $C_{N,O} = 384.4$ kcal-Å^6/mole and $Å_{N,O} = 226{,}490$ kcal-A^{12}/mole.

When the $\cos\phi'$, $\cos^2\phi'$, and $\sin^2\phi'$ (where ϕ' is the dihedral angle between the amide and α-protons [$\phi' = |240° - \phi|$]) are averaged over Map B, the following values were obtained: –0.762, 0.632 and 0.367, which leads to $\langle J_{N\alpha}\rangle = 6.63$ Hz ($J_{N\alpha} = 8.9\cos^2\phi' - 0.9\cos\phi' + 0.9\sin^2\phi'$) (*26*) for the random coil polymers. The 3×3 matrix T_i transforms a vector from the coordinate system along the virtual bond $i + 1$ to the corresponding coordinate system along the virtual bond i. The virtual bond connects adjacent C^α atoms (see Fig. 8) and its length is invariant to conformation (ϕ, ψ) providing the pep-

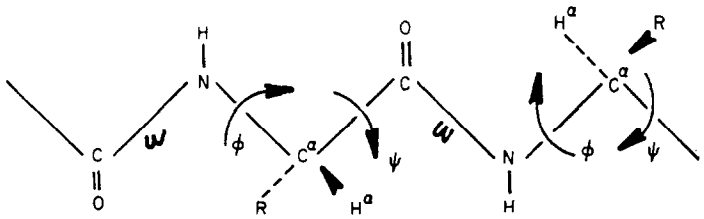


FIG. 8. A schematic representation of an L-peptide chain in the planar zigzag all-*trans* conformation.

tide and ester bonds are rigidly fixed as planar and *trans*. Virtual bonds beginning at C_{ester} and ending at C_{amide} are 3.80 Å in length, while those beginning at C_{amide} and terminating at C_{ester} are 3.7 Å in length. When the matrix T_i is averaged over the energy Maps A and B (Fig. 9), the following results are obtained:

$$\langle T_i\rangle \text{ Map A} = \begin{matrix} .4419 & .4334 & -.6802 \\ -.5330 & -.4406 & -.6013 \\ -.6987 & .6836 & .0409 \end{matrix}$$

$$\langle T_i\rangle \text{ Map B} = \begin{matrix} .3142 & -.0812 & .0520 \\ -.1300 & -.0143 & .0156 \\ .8057 & -.2731 & -0.377 \end{matrix}$$

These averaged matrices may be used to calculate (*27*) the mean-square end-to-end distances $\langle r^2\rangle_0$ or dipole moments $\langle\mu^2\rangle_0$ of the open chain, or acylic random coil depsipeptides. A comparison of the dipole moment and the amide to α-proton coupling constant measured in solution for the acylic hexadepsipeptide with the dipole moment calculated from $\langle T_i\rangle$ and with $\langle J_{N\alpha}\rangle$ Map B = 6.63 Hz would provide a test of the validity of the conformational energy Maps A and B. (The dipole moment of the zwitterionic hexadepsipeptide dissolved in water is a measure of the distance between NH_3^+ and CO_2^- or the end-to-end distance (*28*).

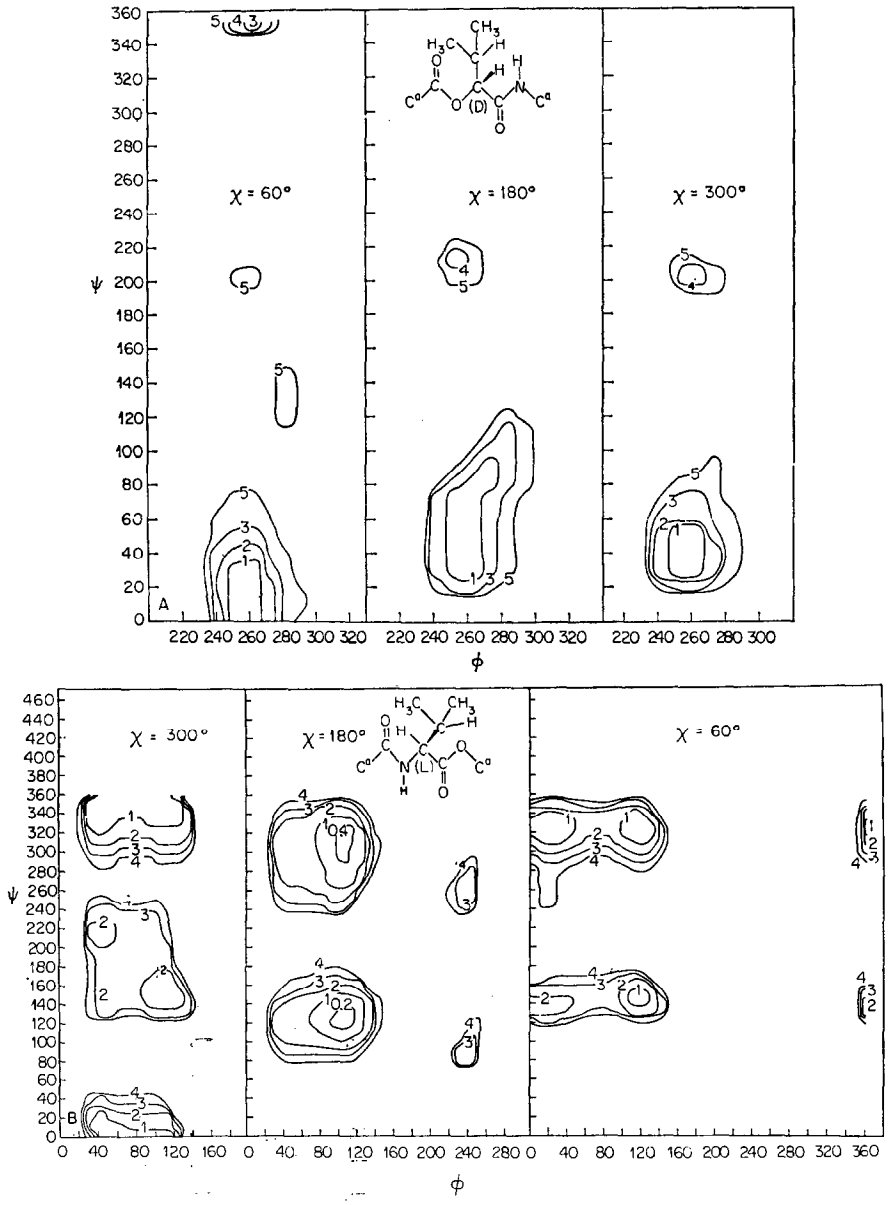
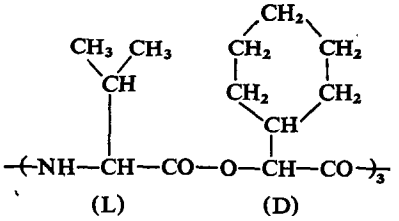


FIG. 9(A, B). Energy maps A (top) and B (bottom).

In search (*29*, *30*) for low-energy cyclic conformations



only those conformations $(\phi, \psi)_{\text{amide(L)}}$ and $(\phi, \psi)_{\text{ester(D)}}$ that lie within the 5.0 kcal mol energy contour of Maps A and B are considered. Since the nmr studies in nonpolar solvents yield $J_{N\alpha}$'s ≈ 9.5 Hz for two of the amide protons and $\leqslant 2.0$ Hz for the third NH, two of the amide protons must be either *trans* ($\phi \leqslant 180°$) or *cis* ($\phi' = 0°$) to their α-protons while the third N–H is gauche ($\phi' = 120°$ or 60° to its α-proton (according to the relation of Bystrov *et al.* (*26*). $J_{N\alpha} = 8.9\cos^2\phi' - 0.9\cos\phi' + 0.9\sin^2\phi'$). Thus, the following conformations were allowed for the amide residues with $J_{N\alpha} \approx 9.5$ Hz:

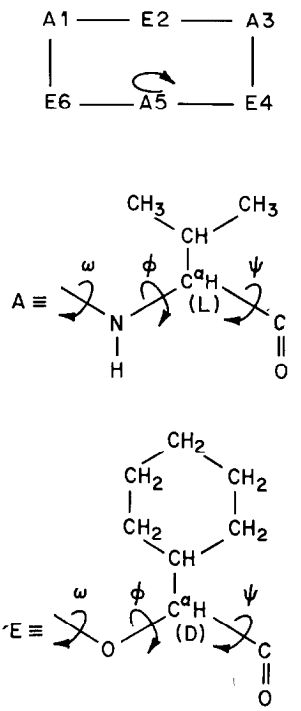


FIG. 10. A schematic representation of the cyclic hexadepsipeptide (L-Val-D-Hex)$_3$, where the arrow indicates movement from N to C$^\alpha$ in the amide residues (A1, A3, A5) and from 0 to C$^\alpha$ in the ester residues (E2, E4, E6).

$\phi = 60°$; =0, 30, 90, 120, 150, 180, 210, 240, 270, 300 and 330°, and $\phi = 240°$; $\psi = 90$, 240, 270, 300 and 330°. $\phi = 0°$; $\psi = 120$, 150, 240, 270, 300°, and $\phi = 120°$; $\psi = 0$, 90, 120, 150, 180, 270, 300, and 330° were allowed for the amide residue with $J_{N\alpha} < 2.0$ Hz. For the D-ester residues $\phi = 240°$; $\psi = 30$ and 60°, $\phi = 250°$, $\psi = 0$, 30, 60, and 210°, $\phi = 260°$; $\psi = 0$, 30, 60, 90, and 210°, $\phi = 270°$; $\psi = 0$, 30, 60, and 90°, $\phi = 280°$, $\psi = 30$, 60, 90, and 120° and $\phi = 290°$; $\psi = 90°$ were chosen as the allowed conformations in the search for cyclic conformations.[4]

[4] The angles of rotation ω, ϕ, ψ defined in Fig. 8 are taken (*31*) as 0° in the *trans* or planar zigzag conformation and are measured in a right-handed sense. A more recently proposed convention (*32*) assigns $\omega = \phi = \psi = 180°$ to the planar zigzag conformation. However, to avoid confusion the most recent convention is not adopted here.

In the initial search for cyclic hexadepsipeptide conformations, all amide and ester bonds were assumed to be planar and *trans*. This search failed to yield a cyclic conformation in which the two amide protons with $J_{N\alpha} = 9.5$ Hz were internally buried or hydrogen bonded, as the temperature dependence of the amide proton chemical shifts in nonpolar solvents indicates (*7*, *9*, *23*, *33–35*).

A second search for cyclic conformations, which allowed the amide bonds to be planar and *cis* as well as *trans*, also failed to yield a conformation consistent with the nmr data. In this search, the geometry proposed by Ramachandran and Venkatachalam (*36*) was adopted for the *cis* peptide bond residues.

A third and final search was conducted for ring conformations. Each of the amide bonds was kept planar and *trans* while the ester bonds were allowed to adopt both the *cis* and the *trans* conformations. The geometry (bond lengths and valence angles) of the *cis* ester bond was assumed to be the same as the *trans* ester bonds (*25*).

Of the cyclic conformations generated in this search, one was found to be most consistent with the nmr data. This conformation [$(\omega, \phi, \psi)_{A1} = 0°, 240°, 240°$; $(\omega, \phi, \psi)_{E2} = 180°, 250°, 0\text{–}30°$; $(\omega, \phi, \psi)_{A3} = 0°, 120°, 0°$; $(\omega, \phi, \psi)_{E4} = 0°, 280°, 120°$; $(\omega, \phi, \psi)_{A5} = 0°, 240°, 240°$; and $(\omega, \phi, \psi)_{E6} = 180°, 260°, 30°$] possesses two intramolecularly hydrogen-bonded amide protons, $(N\text{–}H)_{A1}$ and $_{A5}$ (see Figs. 10 and 11). Both amide protons belong to peptide residues with large $J_{N\alpha}(\phi = 240°, \phi' = 0°)$ and both are hydrogen bonded to the C═O group of the 3 residue. One of these hydrogen bonds [$(N—H)_{A5}—(O═C)_{A3}$] is a seven-membered hydrogen bond of the type discussed by Bystrov *et al.* (*25*). As we have noted (*29*, *37*) previously, this kind of hydrogen bond should be rather weak because of its marked nonplanar nature. However, as can be seen in the photograph of the proposed cyclic depsipeptide conformation, $(N—H)_{A5}$ is partially internally buried. Thus, even if its hydrogen bond to $(C═O)_{A3}$ is weak, one might still expect (*7*, *9*, *23*, *33–35*) its chemical shift to be nearly temperature independent in nonpolar solvents as is observed. In addition, this conformation is further characterized by a sum of residue dipole moments of 12D and a sum of residue energies[5] (including hydrogen bond stabilization) of ca. 8.0 kcal/mole.

The A1 and A5 residues both possess $\phi = 240°$ or $\phi' = 0°$ [$J_{N\alpha} = 8.0$ Hz (calcd), 9.5 Hz (exp)] in the proposed conformation of the depsipeptide, i.e., the amide and α-protons in both residues are *cis* to each other. Recently, Ramachandran *et al.* (*39*) have suggested that only the *trans* arrangement of the amide and α-protons can result in a large coupling ($J_{N\alpha} \geq 6.0$ Hz). However, we believe and have discussed (*40*) the reasons why a large coupling should be and is (*29*, *34*) associated with a *cis* as well as the *trans* arrangement of N—H and C^{α}—H^{α} bonds.

The nmr spectra of the cyclic hexadepsipeptide in deuterochloroform reveal large coupling constants $J_{\alpha-\beta} = 7.5\text{–}10.5$ Hz between the α and β protons of the valine residues. If we assume that only the staggered conformations of the side chains are allowed, this observation leads to the conclusion (*41*) that the α and β protons are predominantly *trans* to each other, i.e., the Val-side chain rotation angle (*31*) χ is predominantly 180° in each of the three residues ($\chi = 0°$, 120°, −120°, corresponds to $\chi = 180°$, 300°, 60° in Ref. *31*). Such an analysis is consistent with the positions of the side chains of the L-valine residues in space-filling models of the conformation assumed by the cyclic hexadepsipeptide in nonpolar solvents.

[5] This does not include the energy of the two *cis* ester bonds (*38*).

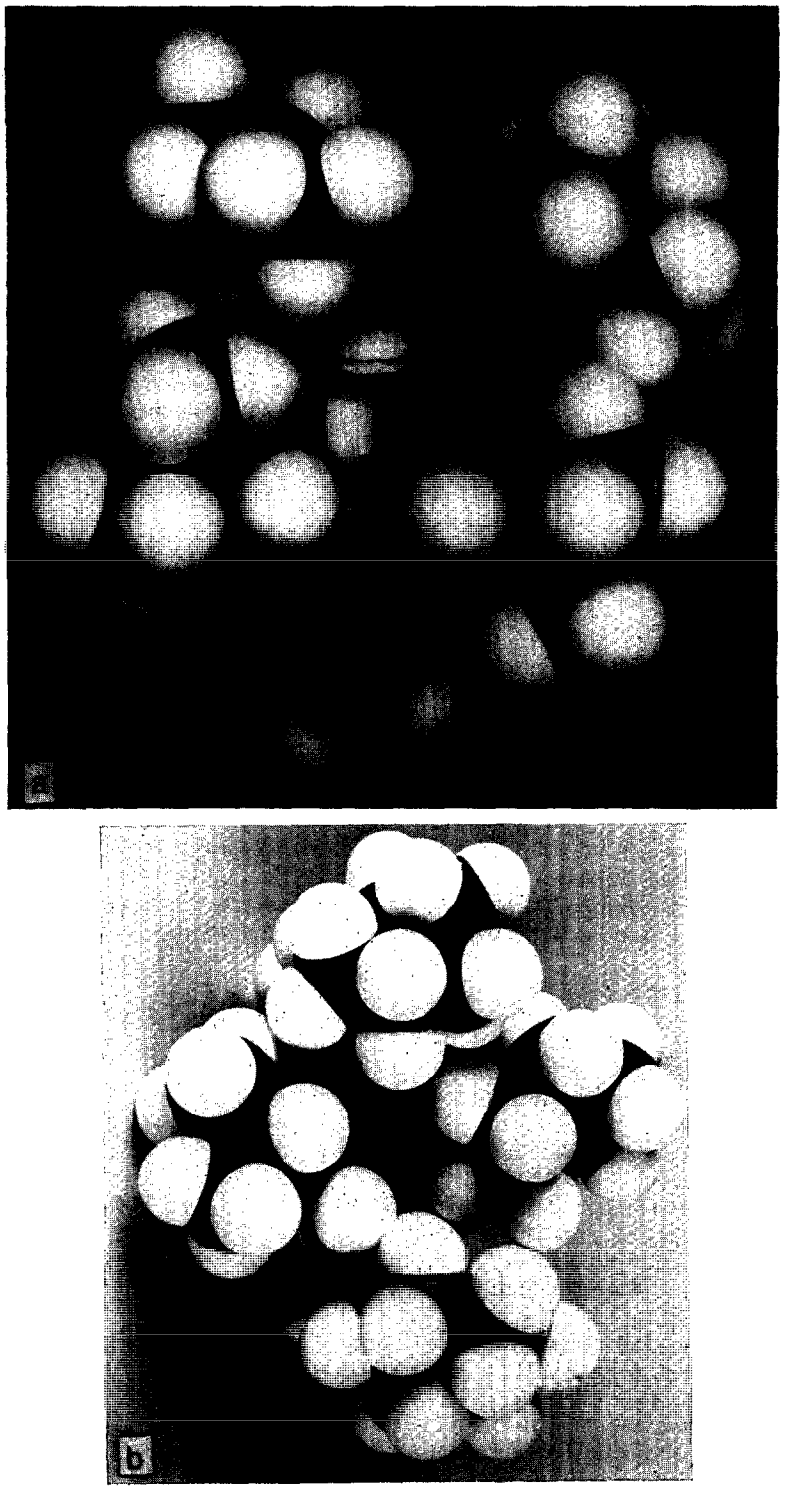


FIG. 11 (a and b). Photographs of molecular space-filling models of the proposed conformation of the cyclic hexadepsipeptide in nonpolar or slightly polar solvents; (a) polar side of the molecule; (b) nonpolar side of the molecule.

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