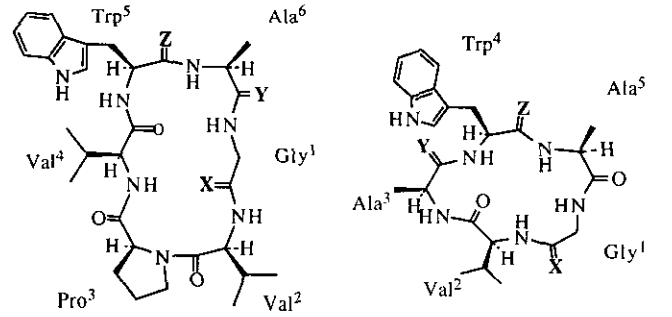
CONFORMATIONAL ANALYSIS OF THIOSEGETALINS BY DISTANCE GEOMETRY CALCULATION

Hiroshi Morita, Young Sook Yun, Koichi Takeya, and Hideji Itokawa*

Department of Pharmacognosy, School of Pharmacy, Tokyo University of Pharmacy & Life Science, 1432-1 Horinouchi, Hachioji, Tokyo 192-03, Japan

Abstract - Three-dimensional structures in DMSO-d_6 of cyclic thiopeptides, thiosegetalins A2, B1 and B2, prepared from segetalins A and B, were determined by distance geometry calculation and restrained energy minimization from NMR data. The backbone structure of thiosegetalin A2, consisting of two β-turns, a β II turn at Trp^5-Ala^6 and a β VI turn at Val^2-Pro^3, retains the backbone conformation of segetalin A, both of which showed estrogen-like activity. Whereas, the backbone conformations of cyclic pentapeptides, thiosegetalins B1 and B2 were different from that of the parent compound, segetalin B. The backbone conformations are important for segetalins to show estrogenic activity. Though thionation is a minimal variation of isosteric replacement, it is a useful conformational modification of cyclic peptides.

In our investigation of bioactive cyclic peptides from higher plants,[1] we reported the structures and estrogenic activity of segetalins A, B, G and H, which have been isolated from the seeds of *Vaccaria segetalis* (Caryophyllaceae),[2-4] and their solution conformations may relate with their estrogenic activity.[5] To investigate relationship between their conformation and activity, we carried out thionation of cyclic peptides, segetalins A and B.[6] Among produced thiosegetalins A and B (Figure 1), thiosegetalin



segetalin A (1)	X=O	Y=O	Z=O
thiosegetalin A1 (3)	X=S	Y=O	Z=S
thiosegetalin A2 (4)	X=S	Y=S	Z=O
segetalin B (2)	X=O	Y=O	Z=O
thiosegetalin B1 (5)	X=S	Y=S	Z=O
thiosegetalin B2 (6)	X=S	Y=O	Z=S

Figure 1 Structures of segetalins A, B, and thiosegetalins A1, A2, B1 and B2

A2, [Gly-1-ψ(CS-NH)-Val-2; Ala-6-ψ(CS-NH)-Gly-1]segetalin A, only showed estrogen-like activity against ovariectomized rats. NMR data including NOE experimental results and temperature effect on

NH protons, suggested that thiosegetalin A2 only retained the backbone conformation like parent compound. The precise conformational investigation by distance geometry (DG) calculation using distance constraints from phase sensitive ROESY experiments[7] resulted in confirmation of the backbone conformation of thiosegetalin A2 and conformational variation of thiosegetalins B1 and B2.

We describe here the conformational preference of thiosegetalins A2, B1 and B2 in DMSO-d_6 solution by a DG - molecular dynamics procedure using distance constraints, and conformational variation of thiosegetalins by thionation.

CONFORMATIONAL ANALYSIS OF THIOSEGETALIN A2

For analysis of the conformational preference of thiosegetalins, it is useful to use a computational method, which does not depend on the starting structures. In the ROE relationship of thiosegetalin A1 in DMSO-d_6, unfortunately no sufficient correlations were observed. Especially, the presence of broad backbone proton resonances and the lack of ROEs around Ala^6 and Gly^1 residues showed a high flexibility of the thiosegetalin A1, which does not permit the solution study of the conformational behavior of the thiosegetalin A1 in DMSO-d_6. For thiosegetalin A2, DG calculation was performed using 20 distance constraints derived from a phase sensitive ROESY experiment. The ROE relationship was shown in Figure 2. Interatomic distances were calculated from the integrated volumes of the ROESY cross peaks and classified into three ranges, 1.8 - 2.5, 1.8 - 3.5 and 1.8 - 5.0 Å, corresponding to strong, medium and weak ROEs, respectively. The distance constraints of the positions of the lack of stereospecific assignments were relaxed by means of the pseudoatom corrections (+1.0 Å for methylene hydrogens and +1.5 Å for methyl hydrogens). Because of the presence of one *cis* amide bond and five *trans* amide bonds, torsional constraints for amide bonds were also used. No hydrogen bonding constraints were used.

The initial 165 structures for thiosegetalin A2, satisfying the experimental restraints, were embedded by DG calculations. Structural calculations were carried out using simulated annealing (SA) protocol with the program SYBYL,[8] and the produced conformers were then subjected to restrained energy minimization with the AMBER all-atom force field.[9,10] In SA simulation, each system was equilibrated for 5000 fs in a thermal bath at 800K, and thereafter successively for 2700 fs, the temperature was decreased 54 times until a final temperature of 100K was reached. Each frozen conformation was finally minimized.

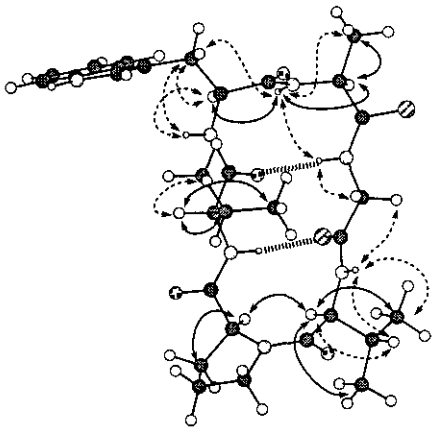


Figure 2. Proposed conformation of thiosegetalin A2 in solution. Arrows show strong ROE relationship and broken arrows show medium or weak ROE relationship. Two thick broken lines represent intramolecular hydrogen bonds.

The results were summarized in Table 1. 29 structures were selected among 165 structures generated for thiosegetalin A2 (pairwise RMSD for the backbone heavy atom is less than 1.00 Å). The average RMSD of the restraint violations was 0.002 Å. Figure 6 shows the mean structure of the backbone heavy atoms.

Comparison between the heavy atoms in each conformer and the average structure gave an average RMSD of 1.66 Å. The mean structure takes two β-turns, one is type II at Trp^5-Ala^6 and the other β VI turn at Val^2-Pro^3. The amide protons of Gly^1 and Val^4 participate in intramolecular hydrogen bonding between Gly^1-NH and Val^4-CO (N-H...O 2.011Å, 132.6°), and between Val^4-NH and Gly^1-CS (N-H...S 2.929Å, 162.7°), which are corresponding to the results of temperature dependence on NH hydrogens.[6] The above solution conformational properties of thiosegetalin A2 confirm our previous findings to exist in similar conformation to that of segetalin A.[6] Stabilization of the backbone conformation of segetalin A by transannular hydrogen bonds may result in unchangeness of its conformation.

Table 1. Results of distance geometry calculations for thiosegetalins A2, B1 and B2

Structural parameters	thiosegetalin A2	thiosegetalin B1	thiosegetalin B2
No. of constraint			
distance	20	28	30
chiral	6	5	5
torsion	6	5	5
No. of calculated conformer	165	327	302
No. of converged conformer[a)]	29	49	36
Mean energy (kcal/mol)	57.8036 (1.5217)	43.0855 (1.6291)	70.9563 (2.1350)
Mean RMS ROE	0.002	0.012	0.011
RMSD for backbone heavy atoms of mean structures (Å)	0.77 (0.20)	0.53 (0.10)	0.39 (0.13)

a) the number of the produced conformers whose pairwise RMSD for backbone heavy atoms is less than 1.00 Å.

CONFORMATIONAL ANALYSIS OF THIOSEGETALINS B1 AND B2

In the case of thiosegetalins B1 and B2, the DG calculations, followed by the SA calculations, were carried out according to the above conditions. The results were shown in Table 1. The ROE relationships used in the DG calculations were summarized in Figures 3 and 4. 49 structures among 327 structures generated by the DG method for thiosegetalin B1, and 36 structures among 302 structures for thiosegetalin B2 were well converged (pairwise RMSD for the backbone heavy atom is less than 1.00 Å). The average conformers of them, followed by energy minimization, were shown in Figure 6. Mean RMS ROE of the restraint violations was 0.01 Å for both compounds. The overall heavy atomic RMSDs between the individual structures and the mean coordinate positions are 1.76 Å for thiosegetalin B1 and 1.77 Å for thiosegetalin B2. The mean structure of thiosegetalin B1 shows one short contact between Val^2-NH and Trp^4-CO (N-H...O 3.712 Å, 130.3°) constructing a type II β-turn at Ala^5 - Gly^1 position, and one intramolecular hydrogen bond between Trp^4-NH and Val^2-CO (N-H...O 1.803 Å, 148.3°) constructing a γ-turn at Val^2 - Ala^3 - Trp^4 position. On the other hand, that of thiosegetalin B2 shows two intramolecular hydrogen bonds between Ala^5-NH and Val^2-CO (N-H...O 2.467 Å, 163.1°) constructing a type I β-turn at Ala^3 - Trp^4, and between Val^2-NH and Ala^5-CO (N-H...O 1.852 Å, 151.4°) constructing a γ-turn at Ala^5 - Gly^1 - Val^2 position. These intramolecular hydrogen bonding hydrogens were also corresponding to the assumption by temperature dependence study in our previous paper.[6]

It is interesting that the above two cyclic thiopentapeptides possess different backbone conformation each other. In addition, these two thiosegetalins B1 and B2 take different backbone conformations from that of the parent peptide, segetalin B. That is to say, thionation of segetalin B resulted in the conformational change of backbone structures and lack of estrogenic activity. In this way, it is useful method for thionation to change backbone conformation of cyclic peptides.

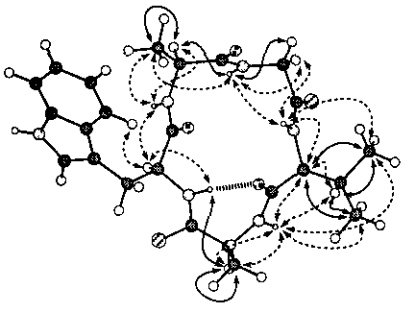


Figure 3. Proposed conformation of thiosegetalin B1 in solution. Arrows show strong ROE relationship and broken arrows show medium or weak ROE relationship. A thick broken line represents an intramolecular hydrogen bond

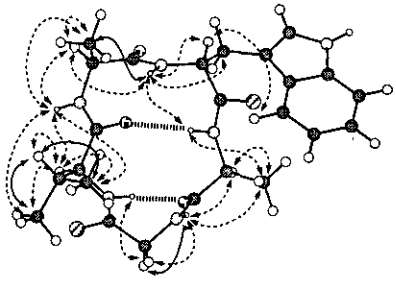


Figure 4. Proposed conformation of thiosegetalin B2 in solution. Arrows show strong ROE relationship and broken arrows show medium or weak ROE relationship. Two thick broken lines represent intramolecular hydrogen bonds.

CONFORMATIONAL CONVERSION OF CYCLIC PEPTIDE BACKBONE BY THIONATION

It is known that the hydrogen donor activity of the nitrogen next to the thiocarbonyl group is increased, comparing to that of an amide bond.[11] Therefore, thionation may result in the conformational conversion of peptide backbone. A few examples were reported about influence of secondary structures by the introduction of a thioamide in cyclic peptides.[12] Conformational conversion for thiosegetalins B1 and B2 are summarized in Figure 5. Segetalin B takes type II-like β-turn at Trp-Ala in DMSO-d_6 in spite of steric interactions of the carbonyl oxygen in Trp with the side chain in Ala.[13] In thiosegetalin B2, conformational conversion may be occurred by the increased steric effect between the thioamide sulfur atom in Trp and the side chain in Ala. All of the amide hydrogens next to the thiocarbonyl group in thiosegetalins B1 and B2 are involved in intramolecular hydrogen bonding. Though thionation is a minimal variation of isosteric replacement, it is a useful conformational modification of cyclic peptides, which might be derived by increased donor potential of the amide hydrogen and/or increased acceptor potential of thioamide carbonyl.

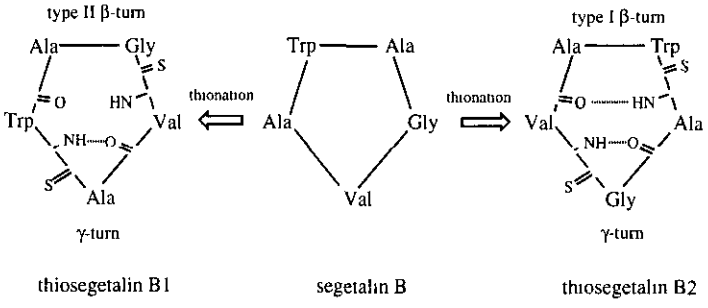
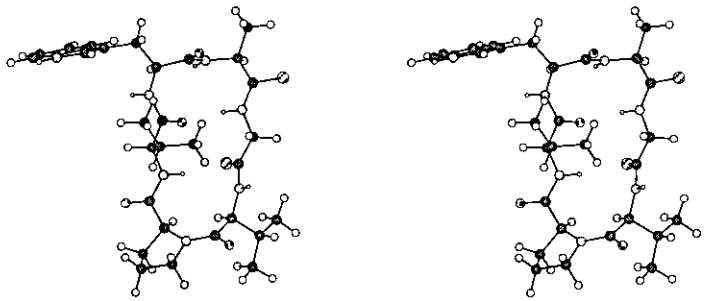
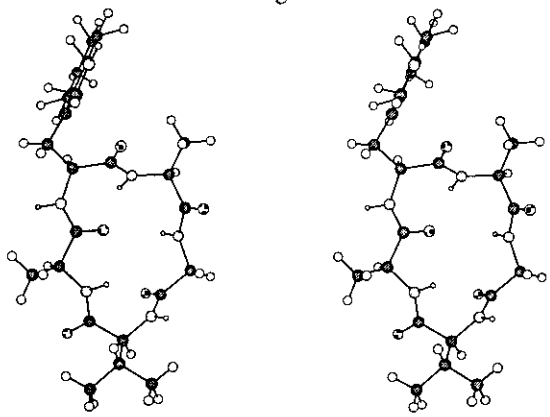


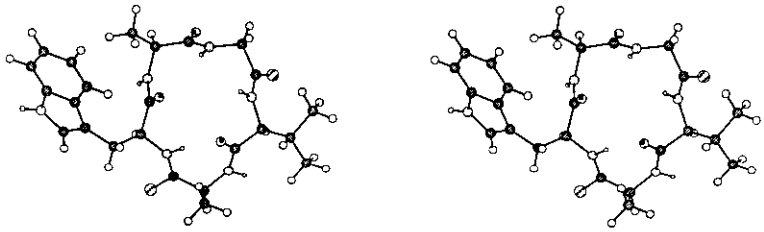
Figure 5. Conformational conversion of thiosegetalins B1 and B2; Dashed lines represent intramolecular hydrogen bonds.



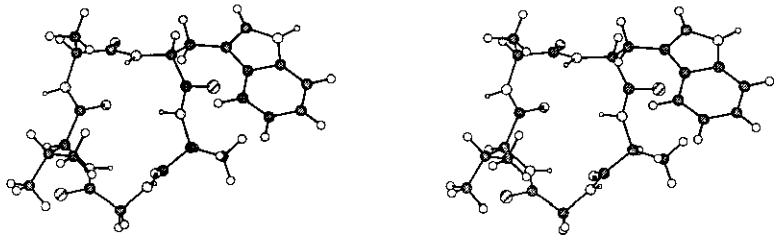
thiosegetalin A2



segetalin B



thiosegetalin B1



thiosegetalin B2

Figure 6. Stereoscopic views of the structures of thiosegetalins A2, B1, B2 obtained by DG calculations, and that of segetalin B.

EXPERIMENTAL

Materials. - Thiosegetalins A2, B1 and B2 were prepared according to the previous procedure.[6]

NMR. - NMR spectra were recorded on Varian Unity 400 spectrometers. Each 10 mg of thiosegetalins A2, B1 and B2 in a 5 mm tube (0.5 mL of DMSO-d_6, degassed) was used for the homonuclear measurements. The spectra were recorded at 300 K. Phase sensitive ROESY experiments were made with a mixing time of 200 msec.

Computational Methods. - Computer modeling and all calculations were carried out using the molecular-modeling software package SYBYL ver. 6.22 (Tripos, Inc., St. Louis, MO) on an IRIS 4D computer. Molecular mechanics and SA calculations were performed with the AMBER all-atom force field.[9, 10] The dielectric constant (ε) was assumed to be proportional to the interatomic distances (r) as ε=r. Solvent molecules were not included in the calculations. The ROE relationships shown in Figures 2, 3 and 4 were taken into account in the calculations of the constrained minimizations and dynamics, with an extra harmonic term of the form $E=1/2k(d-d^{low})^2$ for $d<d^{low}$, $1/2k(d^{high}-d)^2$ for $d^{high}<d$ and $E=0.0$ for $d^{low}\leq d\leq d^{high}$ added to the force field [k=200 kcal/(mol)(°)^2]. Torsion constraints, with an extra harmonic form of the form $E=1/2k(\omega-\omega^0)^2$ [k=0.01 kcal/(mol)(Å^2)] were also added to the force field. In SA simulation, each system was equilibrated for 5000 fs in a thermal bath at 800 K, and thereafter successively for 2700 fs, the temperature was decreased 54 times until a final temperature of 100 K was reached. Each frozen conformation was finally minimized. Each converged group was selected as those whose pairwise backbone RMSD is less than 1.00 Å. Each energy minimization was carried out until the derivatives became less than 0.01 kcal·mol^{-2}·Å^{-1} using the MAXMIN program.

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REFERENCES

1. Cyclic Peptides from Higher Plants. Part 43, Part 42. H. Morita, A. Shishido, T. Matsumoto, K. Takeya, H. Itokawa, T. Hirano, and K. Oka, *BioMed. Chem. Lett.*, 1997, **7**, 1269.
2. H. Morita, Y.S. Yun, K. Takeya, and H. Itokawa, *Tetrahedron Lett.*, 1994, **35**, 9593.
3. H. Morita, Y.S. Yun, K. Takeya, H. Itokawa, and K. Yamada, *Tetrahedron*, 1995, **51**, 6003.
4. Y.S. Yun, H. Morita, K. Takeya, and H. Itokawa, *J. Nat. Prod.*, 1997, **60**, 216.
5. 38th SYMPOSIUM on THE CHEMISTRY OF NATURAL PRODUCTS, SENDAI, SYMPOSIUM PAPERS, pp. 289-294.
6. H. Morita, Y.S. Yun, K. Takeya, H. Itokawa, and O. Shirota, *Bioorg. Med. Chem.*, 1997, **5**, 631.
7. A.A. Bother-By, R.L. Stephens, J. Lee, C.D. Warren, and R.W. Jenanloz, *J. Am. Chem. Soc.*, 1984, **106**, 811.
8. Molecular-modeling software SYBYL ver. 6.22 (Tripos, Inc., St. Louis, MO)
9. S.J. Weiner, P.A. Kollman, D.A. Case, U.C. Singh, C. Ghgio, G. Algona, S. Profeta, Jr, and P. Weiner, *J. Am. Chem. Soc.*, 1984, **106**, 765.
10. S.J. Weiner, P.A. Kollman, D.T. Nguyen, and D.A. Case, *J. Comput. Chem.*, 1986, **7**, 230.
11. E.P. Dudek and G. Dudek, *J. Org. Chem.*, 1967, **32**, 823.
12. H. Kessler, A. Geyer, H. Matter, and M. Kock, *Int. J. Peptide Protein Res.*, 1992, **40**, 25.
13. H. Morita, Y.S. Yun, K. Takeya, and H. Itokawa, *Tetrahedron*, 1995, **51**, 5987.

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