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Molecular modeling of 5-desacetylaltohyrtin A, a spongean cytotoxic macrolide

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Abstract—The solution structure of 5-desacetylaltohyrtin A, an extremely cytotoxic macrolide isolated from a marine sponge, was analyzed by NMR and restrained molecular dynamics. The average value of pairwise RMSD for the backbone (from C_1 to C_{43}) of the 10 lowest energy structures was 0.50±0.22 Å. The stereostructure of C_{14}–C_{16} in 5-desacetylaltohyrtin A was further verified by restrained molecular dynamic calculation using incorrect chiral restraints.

1. Introduction

In 1993, we isolated a new class of extremely cytotoxic macrolides named altohyrtins A (**1**), B (**2**) and C (**3**) and 5-desacetylaltohyrtin A (**4**) from the Okinawan marine sponge *Hyrtios altum* on the basis of bioassay-guided separation.[1] Independently Fusetani and his group have isolated an analogous compound, cinachyrolide A (**5**), from a marine sponge of *Cinachyra* sp.,[2] and Pettit and his group have also isolated spongistatins 1 (**6**) –9 from marine sponges of *Spongia* sp.[3] and *Spirastrella spinispirulifera*.[4] These macrolides were characterized as having the same 42-membered macrolactone ring, two spiroketals, two tetrahydropyranes, and a halogen atom, and they exhibit extremely potent cytotoxic activities against cultured tumor cells. Altohyrtins had 24 chiral centers. We have elucidated the absolute stereostructures of altohyrtins (**1**–**4**) on the basis of detailed NMR analysis, application of a modified 2-methoxy-2-(trifluoromethyl)-phenylacetic acid (MTPA) method to the hexa-MTPA ester, and application of a CD exciton chirality method. The relative stereostructures of two spiroketal parts and two tetrahydropyrane parts each for both cinachyrolide A (**5**) and spongistatins (e.g. spongistatin 1 (**6**)) have also been deduced independently on the basis of NOESY analysis. However, there was no evidence for the optical relationship between each partial structure in cinachyrolide A and spogistatins. Accordingly, the stereostructure from C_{14} to C_{16} in cinachyrolide A has not been defined and the stereostructure of C_{14}–C_{16} proposed for spongistatins was in conflict with that of altohyrtin A (**1**) (Chart 1).

In 1997, the first total syntheses of altohyrtin C by Evans et al.[5] and altohyrtin A by Kishi et al.[6] were performed, and the absolute stereostructures of altohyrtins proposed by us were unequivocally confirmed. The NMR data for cinachyrolide A and spongistatins were closely similar to those of altohyrtins. We proposed that spongistatins 1, 2 and 3 are identical with altohyrtins A, C and 5-desacetylaltohyrtin A, respectively, and cinachyrolide A seems to be a 15-desacetyl analogue of altohyrtin A.[7] So far, more than 40 synthetic studies of altohyrtins as challenging and complex targets have been reported. Altohyrtins (**1**, **2**, **3** and **4**) exhibit extremely potent cytotoxic activities against KB (IC_{50} being 0.01, 0.02, 0.4 and 0.3 ng ml^{-1}) and L1210 (IC_{50} being 0.1, 0.03, 1.3 and 2.3 ng ml^{-1}) cell lines, respectively. As for the mechanism of cytotoxicity of these macrolides, spongistatin 1 has been defined to inhibit microtubule assembly by binding to the vinca alkaloid site of tubulin, which inhibits displacement of GDP bound in an exchangeable site of tubulin. The above-mentioned evidence led us to elucidate the three-dimensional stereostructures of altohyrtins (**1**–**4**) by molecular dynamics calculation on the basis of interproton distance restraints.[7] In this paper, we describe the details of the molecular modeling study of 5-desacetylaltohyrtin A (**4**).

2. Results and discussion

The three-dimensional structures of 5-desacetylaltohyrtin A (**4**), which satisfy the NOE restraints, were constructed by restrained molecular dynamics using NMRchitect-Discover software package (Molecular Simulations Inc. (MSI)) with consistent valence force field (CVFF).[8] In order to obtain the larger number of NOE restraints required for restrained molecular dynamics, we measured the NOESY spectrum of 5-desacetylaltohyrtin A (**4**) in d_6-DMSO at 20°C to

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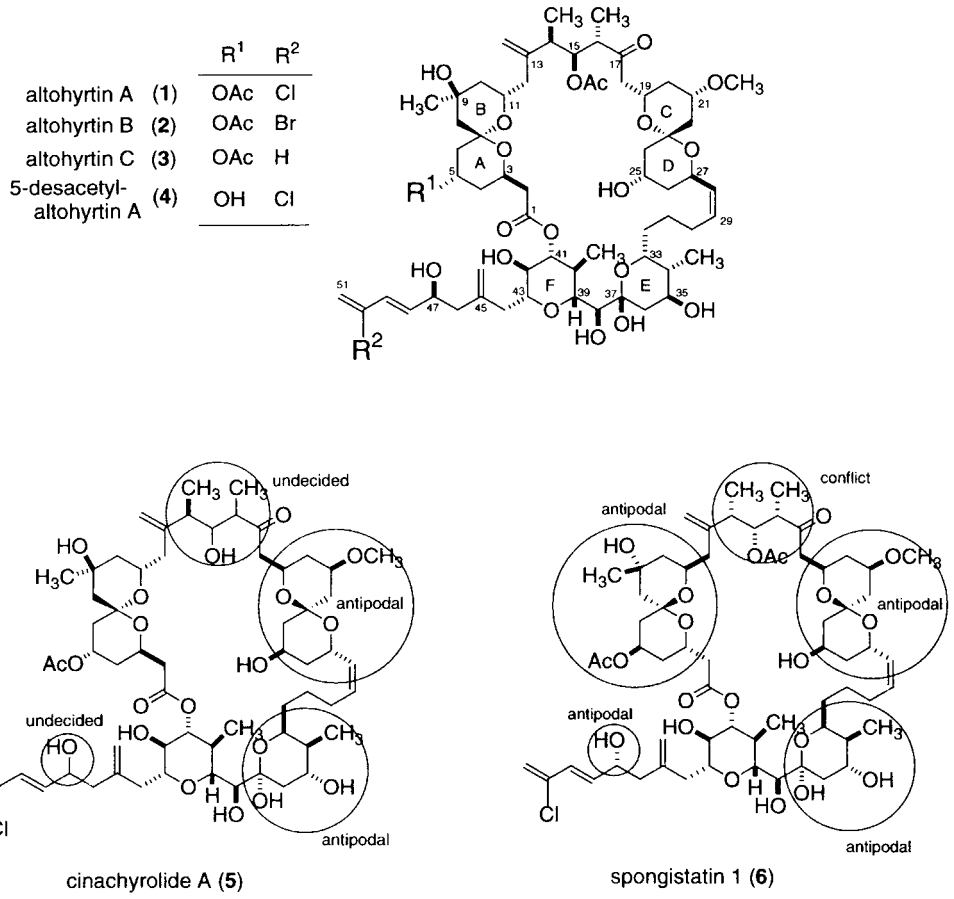

Chart 1.

observe 322 NOE cross peaks. 5-Desacetylaltohyrtin A (**4**) has a total of 17 methylene groups. The assignment of each proton signal of the methylene groups in **4** was performed on the basis of the coupling constant, which was obtained by DQF-COSY (double quantum filtered chemical-shift correlated spectroscopy) analysis, and the relative strength of the NOE cross peak. The unassignable C_{51} *exo*-methylene protons and each methyl proton were treated as pseudo atoms.[9] NOEs were classified into five classes depending on their intensities, and then were translated into distance restraints (upper bound of distance: 2.8, 3.5, 4.0, 5.0 and 6.0 Å). The three $^3J_{HH}$ coupling constants for H_{14}–H_{15}, H_{15}–H_{16} and H_{38}–H_{39}, were converted to broad dihedral angles and were introduced into restrained molecular dynamic calculation as angular restraints. Furthermore, each chiral center was treated as a chiral restraint. A total of 322 distance restraints were used for the restrained molecular dynamic calculation, which was carried out in accordance with the protocol[10] of random array of the atoms-variable force constant (RA-VFC) method.[11] 100 differently randomized structures were generated. 54 of them were acceptable structures having no distance restraint violation (greater than 0.4 Å). The average value of pairwise RMS (root-mean-square) distance deviation for the backbone atoms (from C_1 to C_{43}) among the 10 lowest energy structures was 0.50±0.22 Å. The lowest energy structure and the superimposed 10 lowest energy structures are shown in Fig. 1A and B, respectively. The 10 lowest energy structures for the ring portion (from C_1 to C_{43}) resulted in good convergence to that of the lowest energy structure. Two spiroketals and two tetrahydropyranes seem to be helpful to hold a 42-membered macrolactone ring in **4**. It is interesting to note that 5-desacetylaltohyrtin A (**4**) has a solution structure in which the C_{16}-methyl group and the C_{40}-methyl group are sterically in close proximity.

It is very difficult to elucidate the relative stereostructure of the chain part of a cyclic compound like the C_{14}–C_{16} part in 5-desacetylaltohyrtin A (**4**) under insufficient conditions only for the coupling constant and NOE, which was not classified depending on its intensity. In fact, the Pettit group[3,4] presented the wrong stereostructure for the C_{14}–C_{16} part in spongistatin 1 (**6**). In this molecular modeling, the initial structures are built with completely randomized coordinate by RA-VFC method, and the constraints of the covalent bond, van der Waals radius, and dihedral angle are reduced at the beginning of molecular dynamics. Furthermore, each NOESY cross peak is classified into five classes on the basis of its intensity and translated to a distance restraint. Accordingly, the constructed structures by molecular dynamics are expected to converge to the structure having the correct configurations. Then, to verify the validity of the molecular modeling calculation as a method for determining the relative stereostructure, we further analyzed the stereostructure of the C_{14}–C_{16} part in **4** by molecular modeling calculation.

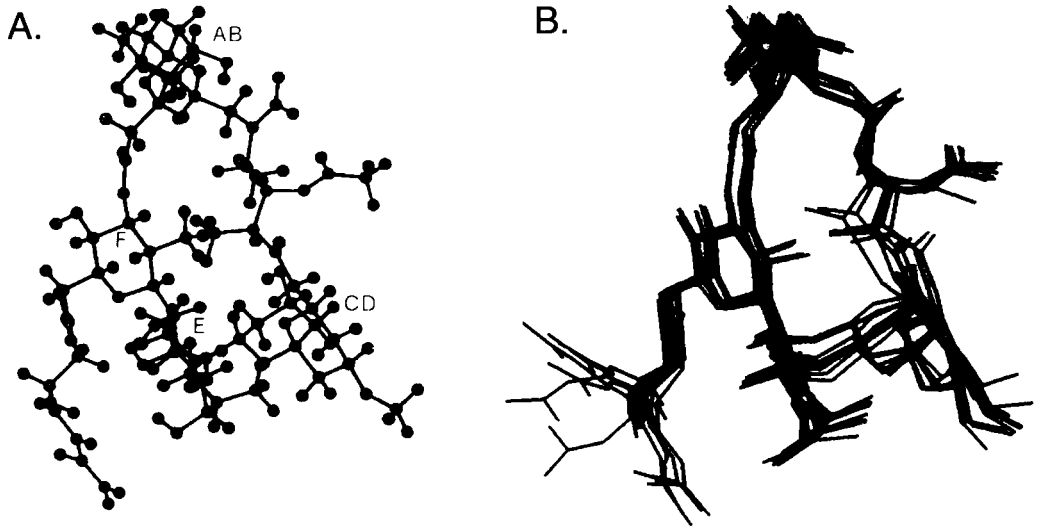

Figure 1. Solution structure of 5-desacetylaltohyrtin A (**4**) calculated by restrained molecular dynamic calculations. (A) The lowest energy structure of **4** as a ball-and-stick model. (B) The superimposed 10 lowest energy structures of **4**. Structures are shown as a wire frame model of heavy atoms (carbons, oxygens and a chlorine).

Table 1. Summary of restrained molecular dynamic calculations for 5-desacetylaltohyrtin A (**4**)

Chirality	E_{total}Ave. (kcal mol^{-1})	RMSD (Å)	Distance RMS viol. (Å)	Dihed. RMS viol. (deg.)	No. of acceptable structures (/100)
14*R*–15*R*–16*R*	166.57±2.43	0.68±0.65	0.174	No violation	32
14*R*–15*R*–16*S*	168.59±2.26	0.89±0.59	0.161	8.79	0
14*R*–15*S*–16*R*	156.05±1.66	1.46±0.49	0.150	6.48	0
14*R*–15*S*–16*S*	**139.43±1.70**	**0.50±0.22**	**0.148**	**No violation**	**54**
14*S*–15*R*–16*R*	173.78±1.46	1.42±0.48	0.176	2.77	25
14*S*–15*R*–16*S*	174.05±1.70	0.86±0.40	0.206	8.59	1
14*S*–15*S*–16*R*	176.80±3.42	1.25±0.55	0.210	14.47	0
14*S*–15*S*–16*S*	164.64±1.62	0.77±0.49	0.178	9.62	3

E_{total} Ave., RMSD, distance RMS viol. and dihed. RMS viol. were obtained from the 10 lowest energy structures and all RMSD were calculated for the backbone atoms (C-1 to C-43). Acceptable structures were chosen with the criteria as follows: (i) no distance restraint violation (greater than 0.4 Å), (ii) no dihedral angle restraint violation, (iii) no chiral restraint violation.

First, we carried out restrained molecular dynamic calculation of **4** without three chiral restraints for C_{14}, C_{15} and C_{16}. Each of the nine lowest energy structures was shown to have the correct 14*R*,15*S*,16*S* configuration. Next, we carried out the same calculation of **4** using seven other sets of chiral restraints (14*R*,15*R*,16*R*; 14*R*,15*R*,16*S*; 14*R*,15*S*,16*R*; 14*S*,15*S*,16*S*; 14*S*,15*S*,16*R*; 14*S*,15*R*,16*R*; 14*S*,15*R*,16*S*). In these calculations, we used the same protocol as that for the standard calculation using 14*R*,15*S*,16*S* chiral restraints. The results of these calculations are summarized in Table 1. Each of the E_{total} average for the 10 lowest energy structures in the cases of incorrect chiral restraints was shown to have much higher values compared with that of the standard calculation (14*R*,15*S*,16*S*). Among the seven sets of calculations, only the two sets that use 14*R*,15*R*,16*R* and 14*S*,15*R*,16*R* chiral restraints gave 32 and 25 acceptable structures having no distance restraint violation in 100 calculated structures, respectively. In all the seven cases, unacceptable structures were obtained in the 10 lowest energy structures, whereas all the 10 lowest energy structures in the standard calculation using correct chiral restraints were acceptable. Furthermore, in the case of using correct chiral restraints, the lowest value of E_{total} average, RMSD, and RMS violation for distance restraints and the largest number of acceptable structures were obtained in 100 calculated structures. From these findings, molecular modeling calculation might be helpful to determine the relative stereostructure of macrocyclic compounds having a partly undefined chain part.

3. Experimental

3.1. NMR spectroscopy

5-Desacetylaltohyrtin A (**4**) was dissolved into DMSO-d_6 (CEA) at a concentration of 15 mg ml^{-1}. The two-dimensional (2D) NOESY spectrum with 400 ms mixing time was recorded on an ARX 500 spectrometer (Bruker) at 20°C. Data points of 512 (t_1)×512 (t_2) in complex points were acquired with a phase-sensitive mode using the TPPI-States method. The spectral width in both axes was 4000 Hz. The relaxation delay was 2.0 s. The residual water signal was suppressed by a long weak pulse, with nominal power during the relaxation delay and the NOESY mixing delay.

After each transient, two orthogonal phased 500 μs and 1 ms of hard power spin lock pulses were applied in order to facilitate a faster recovery of steady state. Such time domain data were zero-filled once and a π/3 shifted squared sine bell was multiplied in both axes, then Fourier transformed up to 1024 (f_1)×1024 (f_2) real points. The two-dimensional (2D) DQF-COSY spectrum was measured on an Alpha 600 spectrometer (JEOL) at 20°C. Data-points of 512 (t_1)×1024 (t_2) in complex points were acquired with a phase-sensitive mode using the States method. The spectral width in both direct and indirect axes was 5000 Hz. The relaxation delay was 2.0 s. The residual water signal was suppressed by the DANTE pulse train series with nominal power during the relaxation delay. Such time domain data were zero-filled and a π/3 shifted squared sine bell was multiplied in both axes, then Fourier transformed up to 1024 (f_1)×2048 (f_2) real points.

3.2. Restrained molecular dynamic calculation

Restrained molecular dynamic calculations were carried out on an Indy R4400 (Silicon Graphics Inc., 150 MHz clock frequency). During molecular dynamics, maximum force restraints of bond distance and dihedral angle were set at 25 kcal $mol^{-1}A^{-2}$ and 50 kcal mol^{-1} rad^{-2}, respectively. Non-bonded repulsion function was cut off at 10.0 Å. To maintain correct chirality, 24 chiral restraints were applied with 10 kcal mol^{-1} of the force constant apparent for the $F_{kchiral}$ parameter in the NMRchitect-Discover package (MSI). In the first step, an initial structure of **4** was built with a complete random array of atoms. For all energy minimizations the conjugate-gradient method was used and 1500 steps were needed for convergence. Then, simulated annealing was executed for 30 ps at 1000 K using the RA-VCF method.[10,11] While the temperature was cooled down stepwise to 300 K, further 30 ps of dynamics were executed. Then, the obtained structure was again refined by energy-minimization once again. RMSD value and other resulting values shown in Table 1 were obtained using Insight-II (MSI) and in-house C shell and awk programs.

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