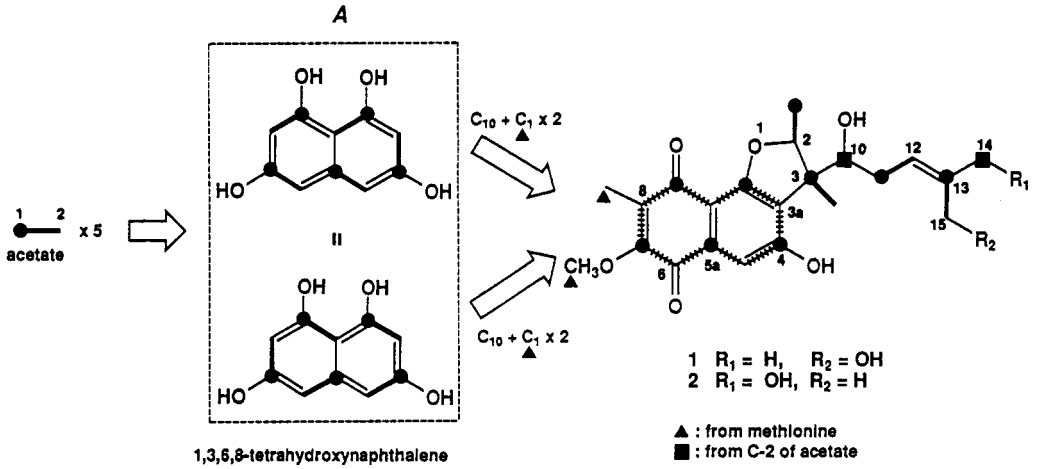
Scheme I. Biosynthetic Pathway of Furaquinocins A (1) and B (2)



This result suggests that the biosynthesis of the naphthoquinone ring may involve two different patterns of acetate arrangement, which is also revealed by the 2D INADEQUATE[4] spectrum of [1,2-$^{13}C_2$]acetate labeled **1**. The furaquinocins are therefore most likely produced through a symmetric intermediate such as 1,3,6,8-tetrahydroxynaphthalene (A; Scheme I), which was also proposed for the biosynthesis of scytalone[5-7] and napyradiomycins.[8] The labeling pattern of the C_{10} unit (a part of the dihydrofuran ring and the side chain: Me-2, C-2, C-3, Me-3, and C-10–C-15) is consistent with biosynthesis involving mevalonate. The ^{13}C NMR signals for C-10 and C-14 appear as singlets and eight additional satellite peaks are observed for the other carbons of the side chain, their J_{CC} values indicating four acetate units for Me-2/C-2, C-3/Me-3, C-11/C-12, and C-13/C-15. The only structural difference between **1** and **2** is the geometry of Δ^{12}-double bond. In each of the ^{13}C spectra of **1** and **2** labeled with [1,2-$^{13}C_2$]acetate, the signal for C-14 (CH_3 in **1** and CH_2OH in **2**) is observed as a singlet and that for C-15 (CH_2OH in **1** and CH_3 in **2**) is coupled with C-13 as shown in Table I. These results imply that both terminal methyl carbons (C-14 and C-15) could be oxygenated and that no *E–Z* isomerization took place during the biosynthetic process.

The ^{13}C NMR spectra of **1** and **2** labeled with [*methyl*-^{13}C]-L-methionine exhibit high incorporations (more than 90%) for two methyl carbons (MeO-7 and Me-8), thereby accounting for the origin of all 22 carbons of **1** and **2**.

It may be worth noting that the carbon for Me-8 does not arise from propionate, which had been suggested when the symmetrical intermediate (A) was postulated in the biosynthesis of the naphthoquinone ring. Concurrent attachment of an isoprenoid side chain and a C_1 unit from methionine onto the polyketide backbone is also interesting. Although a few isoprenoid antibiotics produced by actinomycetes have been reported,[8-11] furaquinocins may be the first example[12] involving a carbon–carbon bond between an inside position of the isoprenoid chain (C-3) and the polyketide nucleus (C-3a).

Acknowledgment. This work was supported, in part, by Grants-in-Aid from the ministry of Health and Welfare and the Ministry of Education, Science and Culture and by funds from the Japan Keirin Association.

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(5) Sankawa, U.; Shimada, H.; Sato, T.; Kinoshita, T.; Yamasaki, K. *Tetrahedron Lett.* **1977**, 483–486.
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Solution and Crystal Structures of (+)-Hitachimycin (Stubomycin)[1]

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Summary: The complete relative and absolute stereochemistry, as well as the solid-state and solution conformations of the antitumor–antibiotic (+)-hitachimycin (stubomycin) (**1**) have been defined via two-dimensional NMR experiments, single-crystal X-ray analysis, and computational methods.

[†]Deceased February 13, 1985.

(1) Dedicated to the memory of Professor Jerry Donohue.

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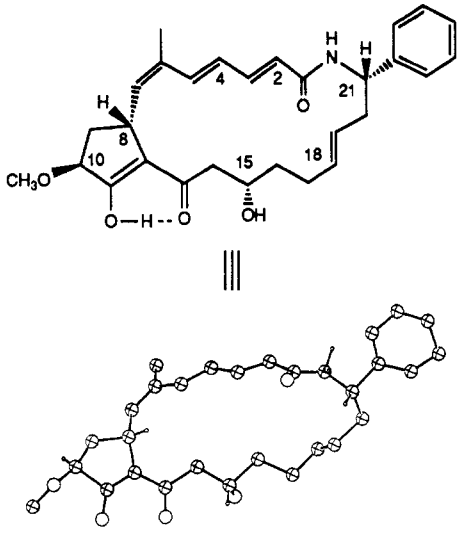
In the early 1980s Ōmura[2] and Umezawa[3] independently isolated the macrocyclic lactam antibiotic (+)-hitachimycin (stubomycin) (1) from an unidentified actinomycetes strain (MK-4927) and from streptomyces (sp KG-2245), respectively. The connectivity of 1 was deduced by Ōmura via a series of ^1H and ^{13}C NMR studies, in conjunction with extensive degradation work,[4] but the relative and absolute stereochemistry remained undefined. Our interest in hitachimycin stemmed from both its novel architecture and its reported cytotoxicity against a variety of tumor cell lines.[5] Herein we report the relative and absolute stereochemistry of hitachimycin as defined by two-dimensional proton correlated NMR spectroscopy (COSY) and single-crystal X-ray analysis. Importantly, this work, coupled with NOE data and computational results, also permits definition of the solution conformation of 1.



(+)-Hitachimycin 1

To resolve the issue of relative stereochemistry, a complete set of X-ray diffraction data was collected; refinement yielded structure 1 with an associated R value of 13.1%.[6] The high value of R derived from the unexpected inclusion of two disordered molecules of chloroform in the unit cell, as well as the poor quality of the crystal. Nevertheless this analysis, in conjunction with Ōmura's isolation of the degradation product S-(+)-β-phenyl-β-alanine methyl ester, permitted assignment of the absolute stereochemistry 8R, 10S, 15S, and 21S. The enolic form illustrated in 1 also derived from the X-ray analysis.

To provide additional support for structure 1, and also to elucidate the solution conformation of the 19-membered macrocyclic ring, we initiated 2D NMR (500 MHz) and computational studies.[7] First, we addressed the question of conformational homogeneity. As established in the peptide area, a single conformation in solution will express

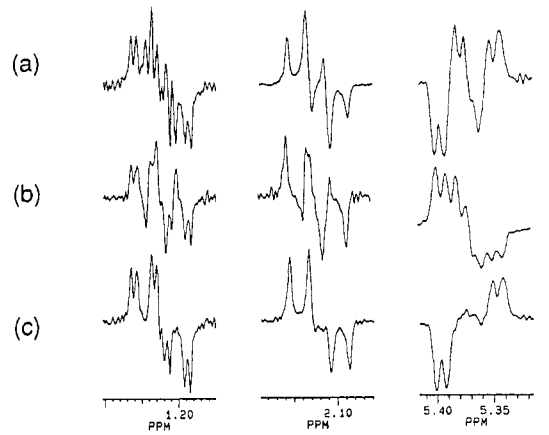


Figure 1. The top traces (a and b) in each example are cross-peak sections created by the Bruker DISCO routine. Trace c in each results from subsequent addition or subtraction of the top two traces.

characteristic NMR behavior.[8] Specifically, the presence of a predominant conformation was indicated by the large chemical shift differences (ca. 0.15–0.40 ppm) between the diastereotopic hydrogens at C(14), C(16), C(17), and C(20), as well as the downfield resonance of the C(20)-allylic hydrogen (δ 2.75 ppm).[9] In addition, chemical shifts in the ^{13}C and ^1H spectra and coupling constants in the latter underwent no relevant change upon increase of solvent polarity or variation of temperature over a 110 °C range (−50 °C → 60 °C).[10] Low temperature did slow the exchange of the vinylogous acid and secondary hydroxyl hydrogens, whereupon these previously unobserved resonances appear as broad singlets at 12.9 and 3.7 ppm, respectively. Finally, the larger than average vicinal couplings provided additional support for the existence of conformational homogeneity.

Confident that the experimentally derived coupling constants would define the solution conformation, we determined all nonaromatic ^1H coupling constants by employing both 1D and 2D NMR techniques.[11] Of particular

(2) Ōmura, S.; Nakagawa, A.; Tanaka, Y. In *Trends in Antibiotic Research*; Umezawa, H., Ed.; Japan Antibiotics Research Association: Tokyo, 1982; pp 135–145.

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(6) Although suitable for tentative assignment of the relative stereochemistry, this R value is about 50% larger than currently acceptable.

(7) All NMR work was carried out with a Bruker AM 500 spectrometer.

(8) (a) Of the characteristics described by Kessler for conformational homogeneity,[8b] those reasonably extended to non-peptide systems include: (i) large chemical shift differences between diastereotopic geminal protons; (ii) vicinal coupling constants that differ greatly from the "mean value" of ca. 7.5 Hz; and (iii) little or no change in coupling constants following change of solvent and/or temperature. (b) For a full discussion, see: Kessler, H. *Angew. Chem., Int. Ed. Engl.* **1982**, *21*, 512. Kessler, H.; Bermel, W. In *Methods in Stereochemical Analysis*; Takeuchi, Y., Marchand, A. P., Eds.; VCH: Deerfield Beach, 1986; Vol. 6, p 179.

(9) Jackman, L. M.; Sternhell, S. Application *of NMR Spectroscopy in Organic* Chemistry; Pergamon: New York, 1969; Vol. 5, p 172.

(10) At temperatures near −50 °C the conformational mobility of the cyclopentyl unit became apparent, as evidenced by significant changes in the C(8)- and C(9)-hydrogen resonances. No change in the C(10)-hydrogen resonance or the coupling of the C(7)- and C(8)-hydrogens was noted.

(11) For solution conformation studies in other macrocyclic systems, see: (a) Peptidic systems; Bruch, M. D.; Noggle, J. H.; Gierasch, L. M. *J. Am. Chem. Soc.* **1985**, *107*, 1400. Glickson, J. D.; Gordon, S. L.; Pitner, T. P.; Agresti, D. G.; Walter R. *Biochemistry* **1976**, *15*, 5721. Kessler, H.; Bats, J. W.; Griesinger, C.; Koll, S.; Will, M.; Wagner, K. *J. Am. Chem. Soc.* **1988**, *110*, 1033. (b) nonpeptidic systems; Everett, J. R.; Tyler, J. W. *J. Chem. Soc., Perkin Trans. 2* **1987**, 1659. Baker, G. H.; Brown, P. J.; Dorgan, R. J. J.; Everett, J. R.; Ley, S. V.; Slawin, A. M. Z.; Williams, D. J. *Tetrahedron Lett.* **1987**, *28*, 5565. Cellai, L.; Cerrini, S.; Segre, A.; Brufani, M.; Fedeli, W.; Vaciago, A. *J. Org. Chem.* **1982**, *47*, 2652. Arora, S. K.; Kook, A. M. *J. Org. Chem.* **1987**, *52*, 1530. Radics, L.; Incze, M.; Dornberger, K.; Thrum, H. *Tetrahedron* **1982**, *38*, 183. Sugiura, M.; Beierbeck, H.; Bélanger, P. C.; Kotovych, G. *J. Am. Chem. Soc.* **1984**, *106*, 4021. Kam, M.; Shafer, R. H.; Berman, E. *Biochemistry* **1988**, *27*, 3581. For the effect of conformation on chemical reactivity see: Still, W. C.; Galynker, I. *Tetrahedron* **1981**, *37*, 3981. Still, W. C. In *Current Trends in Organic Synthesis*; Nozaki, H., Ed.; Pergamon: New York, 1982; p 233. Still, W. C. *J. Am. Chem. Soc.* **1979**, *101*, 2493.

value in the analysis of the two-dimensional, phase-sensitive COSY experiments[12] was DISCO addition or subtraction of appropriate f-2 slices.[13] This recently developed method reduced the cross-peak multiplicities by a factor of two, thereby solving the propinquity problem of the antiphase peaks and permitting accurate coupling constant measurement (Figure 1).[14,15]

Importantly, the experimentally derived coupling constants showed an excellent correlation (R^2 = 0.95) with those predicted for the X-ray structure.[16] Thus the solid-state conformation appeared to be conserved upon solvation. To evaluate the energies of the solid-state conformation and other possible conformers, an extensive conformational search was undertaken employing both the multiconformer[17] and Monte Carlo[18] technique. By varying the torsion angles about the 15 single bonds of the macrocyclic ring, the multiconformer and Monte Carlo methods generated and then partially minimized over 200 and 3000 conformations, respectively.[19] Futher refinement of the 20 lowest energy conformations from the multiconformer method and the 1430 unique conformations from the Monte Carlo search yielded 10 distinct minima within 2.5 kcal/mol of the global minimum. Both the multiconformer and Monte Carlo methods found the same global minimum; however, the majority of the low energy conformations (i.e., 7) were found by the Monte Carlo technique. Importantly, the global minimum was identical to the X-ray structure except for small differences in transannular distances and appendage positions.[20] Analysis of this conformer using the NMR mode of MacroModel again demonstrated an excellent correlation (R^2 = 0.95) with the experimentally derived coupling constants. Notably, only one of the calculated local minima (δE = 2.51 kcal/mol) exhibited a correlation (R^2 = 0.93) approaching that of the global minimum. This conformer was dismissed as a possible solution structure based upon the incompatibility of its interatomic distances with the experimental NOE data.[21]

To further define the solution conformation, we carried out a series of 1D NOE difference experiments. Important features of this analysis included: (A) the observation of a large NOE (17%) between the C(5)- and C(8)-hydrogens, which served to establish an orthogonal orientation of the C(1–7) olefinic bridge with the cyclopentene ring; (B) the existence of a 5% NOE between the C(16a)- and C(18)-hydrogens, indicative of a staggered orientation of the isolated ethylene unit relative to the C(16)-methylene group; and (C) the presence of two NOE's of similar intensity (ca. 4%) between the C(14)- and C(16)-methylenes, suggestive of an extended (zig-zag) arrangement of the aliphatic bridge. These observations without exception were reflected in the solid-state and computational conformations, and again indicate that the solid-state conformation is present in solution.

In summary, we have defined the complete relative and absolute stereochemistry of hitachimycin and demonstrated that the solution conformation and MM2 global minimum are essentially identical with the solid-state conformation. Importantly, this study illustrates the utility of current NMR techniques for conformational analysis of complex, nonpeptide macrocycles. Of particular value was the DISCO method for the deconvolution of phase-sensitive cross peaks. Finally, we have demonstrated the ability of current computational methods to model successfully the NMR behavior of a macrocyclic system.

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Registry No. (+)-Hitachimycin, 77642-19-4.

Supplementary Material Available: Contour plots of COSY and heteroatom correlation 2D spectra, PANIC simulation, coupling constant and interatomic distance data for the 16 calculated local minima, full crystal structure data, ORTEP plot of the unit cell, variable-temperature ^{13}C data, and a full listing of experimental and calculated coupling constants (21 pages). Ordering information is given on any current masthead page.

(12) Ernst, R. R. *Chimia* **1987**, *41*, 323.

(13) A 4k data set in the f-2 dimension was initially collected. Appropriate cross sections were generated in the AP2D sub-mode using the Bruker DISCO subroutine. This resulted, after inverse Fourier transform and two zero fills, in a retransformed f-2 cross section with ca. 0.2-Hz digital resolution. The cross-peak addition or subtraction was subsequently carried out using the Bruker dual display mode.

(14) Kessler, H.; Müller, A.; Oschkinat, H. *Magn. Reson. Chem.* **1985**, *23*, 844.

(15) To confirm the measured coupling constants, we employed the NMR simulation program PANIC. With no iterative adjustment of the experimental data, we observed a near perfect comparison, indicating that our analysis was correct.

(16) (a) This comparison was made by using the NMR mode of MacroModel. (b) Still, W. C.; Mohamadi, F.; Richards, N. G. J.; Guida, W. C.; Lipton, M.; Liskamp, R.; Chang, G.; Hendrickson, T.; DeGunst, F.; Hasel, W., MacroModel V2.5, Department of Chemistry, Columbia University, New York, NY 10027. (c) For comparison, the calculated coupling constants of diastereotopic hydrogens were matched with the corresponding experimental values to give the best fit.

(17) The multiconformer calculations were carried out employing a torsion angle resolution of 60° and a closure bond window of 5 Å between C(20,21), see: Lipton, M.; Still, W. C. *J. Comp. Chem.* **1988**, *9*, 343.

(18) Chang, G.; Guida, W. C.; Still, W. C. *J. Am. Chem. Soc.* **1989**, *111*, 4379.

(19) The number 3000 did not result from a fully converged Monte Carlo search. However, due to the absence of a restart option in the current version of MacroModel (Batchmin V. 2.6) this number represents the longest period of uninterrupted computer time available (600 hours).

(20) Complete MM2 minimization of the X-ray structure resulted in a conformer identical in energy with that obtained from the multiconformer study.

(21) The high degree of correlation for this conformer arises due to the sinusoidal nature of the Karplus and Karplus-like equations.[8b]