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Solution Structure of (+)-Discodermolide

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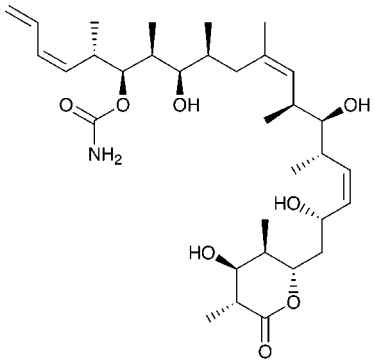
ABSTRACT



(+)-Discodermolide (**1**)

The solution structure of (+)-discodermolide (1) has been determined via 1- and 2-D NMR techniques in conjunction with Monte Carlo conformational analysis. Taken together, the results demonstrate that in solution (+)-discodermolide occupies a helical conformation remarkably similar to the solid state conformation.

In 1990 (+)-discodermolide (**1**) was isolated from the deep sea marine sponge *Discodermia dissoluta* and identified as a potent immunosuppressive agent (Figure 1).[1] The novel structure, in conjunction with the immunosuppressive activity, engendered considerable interest in the synthetic community, culminating to date in seven total syntheses.[2] The subsequent identification of **1** as a potent tubulin stabilizer/anticancer agent that mimics the biological profile of Taxol (**2**)[3] persuaded us to develop a second-generation synthesis capable of delivering (+)-discodermolide (**1**) on a gram scale.[2d] Importantly, (+)-discodermolide (**1**) displays potent activity against multi-drug-resistant (MDR) carcinoma cell lines, including Taxol-resistant lines.[4] More recently, in collaboration with Horwitz, we demonstrated that the combination of (+)-discodermolide and Taxol leads to increased potency against Taxol-resistant cell lines.[5] Thus, the com-

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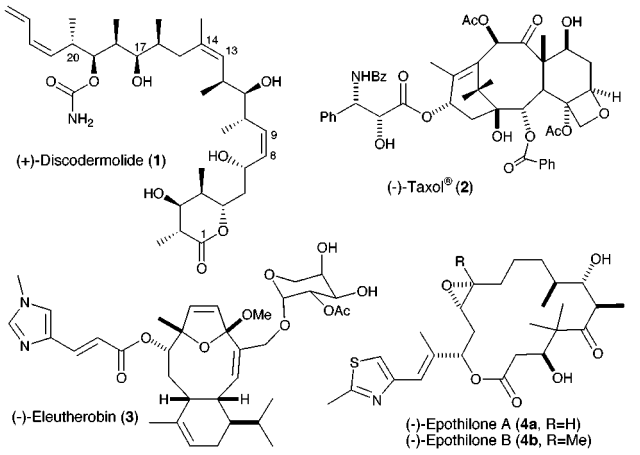

Figure 1. Tubulin-stabilizing natural products.

bination of discodermolide and Taxol constitutes a promising new synergistic cancer chemotherapeutic treatment.[5]

Recently, (+)-discodermolide (**1**) and Taxol (**2**) were reported to share a common tubulin binding site via a common pharmacophore.[6] Other tubulin-stabilizing natural products, including eleutherobin (**3**) and epothilones A and B (**4a**,**b**), have also been proposed to share this pharmacophore.[7] Although Taxol (**2**), eleutherobin (**3**), and the epothilones (**4a**,**b**) have been extensively studied via analog preparation and solution conformational studies,[8] only a modest number of (+)-discodermolide analogs have been reported to date.[9] Moreover, the solution conformation of (+)-discodermolide has not been reported; only the solid state structure[1] and a single theoretical analysis involving a gas-phase density functional study provide information on the conformation of (+)-discodermolide.[10]

To explore the potential of (+)-discodermolide as a lead cancer chemotherapeutic agent, we recently initiated an analog program to define the minimum critical structure for activity.[11] Central to this program is an understanding of the bioactive conformation. We reasoned that an increased understanding of the solution conformational properties of discodermolide might permit the design of new antimitotic agents. Herein we report the solution structure of (+)-discodermolide (**1**) as well as an analysis of structural motifs responsible for this conformational bias.

The structure of (+)-discodermolide (**1**) consists of a linear polypropionate backbone, punctuated by 13 stereogenic centers with 15 rotatable σ bonds and three (*Z*)-olefinic linkages at C(8, 9), C(13, 14), and C(21, 22). A carbamate, δ-lactone, and four hydroxyl moieties further augment the complexity of the structure. Defining conformational minima thus represents a significant challenge.

We initiated our solution studies with the assignment of all non-overlapping hydrogens, including the four hydroxyl hydrogens, via a combination of COSY,[12] TOCSY,[13] and NOESY[14] NMR techniques (500 MHz). The majority of the NMR studies were performed in deuterated acetonitrile (without rigorous dehydration) due to the inherent instability of (+)-discodermolide in both chloroform[1] and dimethyl sulfoxide solutions. We reasoned that the polarity of acetonitrile would mimic the biologically relevant water environment as evidenced by the similarity of the ^{1}H NMR spectrum of (+)-discodermolide in both solvents, and in combinations thereof.

We next performed comprehensive NOESY and ROESY[15] experiments at various mixing times and temperatures. Best results were achieved using the ROESY technique (600 MHz) at room temperature (200 ms spin lock). A total of 38 cross-peaks were resolved, 17 of which represented adjacent hydrogen (i.e., COSY) interactions. The remaining 21 ROESY interactions were employed to generate a distance-constraint file in Macromodel 6.0.[16] This method has been shown to locate effectively conformational minima in flexible systems.[17] To probe thoroughly the conformational potential surface, a 20 000 step Monte Carlo search was performed using the MM2[18] force field, in conjunction with the generalized Born/surface area (GB/SA) water solvent model.[19] Similar searches were performed from different starting geometries to ensure proper convergence. All local minima within 50 kcal of the global minimum were saved and re-minimized. Conformations within 2 kcal of the global minimum (all found several times each) were then overlaid (Figure 2).

Despite the inherent flexibility of the system, the Monte Carlo analysis using the ROE distance constraints provided a convergent array of conformational minima. Evident in all minima were two major turns in the carbon backbone inducing a helical twist to the scaffold. An overlay of the global minimum conformation resulting from the computation and the known crystal structures[1] of (+)-discodermolide (Figure 3A) revealed considerable agreement.[20] Thus, the

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(20) Few ROE interactions were resolved in the δ-lactone region [C(1)−C(6)], which could explain the divergence in this area.

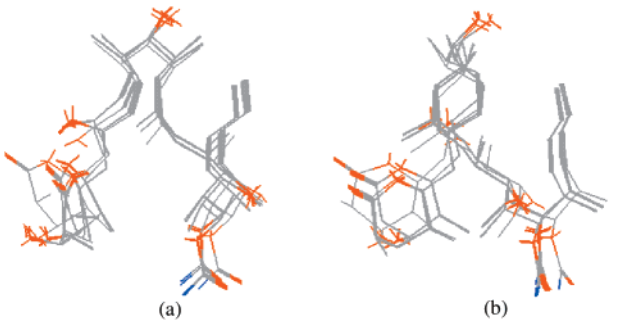

Figure 2. Overlay of conformers within 2 kcal of the global minimum: (a) front view; (b) side view.

solution model proved quite similar to the solid state conformation. Upon further analysis of the solution and solid state conformations, the spatial orientation of the hydroxyl moieties suggested several intramolecular hydrogen bonds (Figure 3B). The C(7) hydroxyl appeared in close proximity to the ring oxygen of the δ-lactone. In addition, the C(3) and C(17) hydroxyl moieties appeared capable of interacting

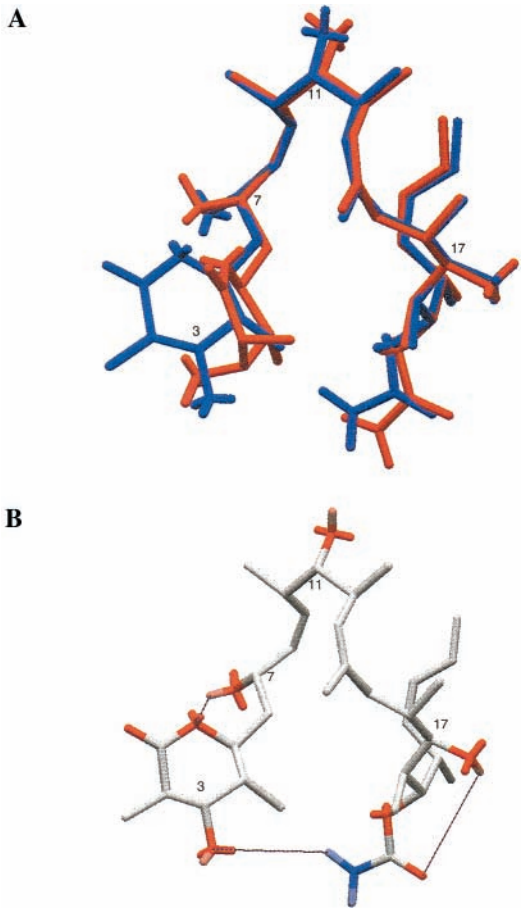

Figure 3. (A) Overlay of computational global minimum (red) and crystal structure (blue). (B) Possible intramolecular hydrogen-bonding interactions.

with the carbamate via long-range hydrogen bonds (>2.6 Å).[21]

To investigate the hydrogen-bonding possibilities, variable temperature and cosolvent titration NMR studies were performed at a concentration of 1.5 mM.[22] Infrared techniques were not utilized due to the coalescence of absorptions in the hydroxyl region. Only the C(11) hydroxyl hydrogen had a greater temperature dependence (−0.006 ppm/K), when the ^{1}H NMR spectra were determined as a function of temperature (Figure 4A). Three out of four hydroxyl

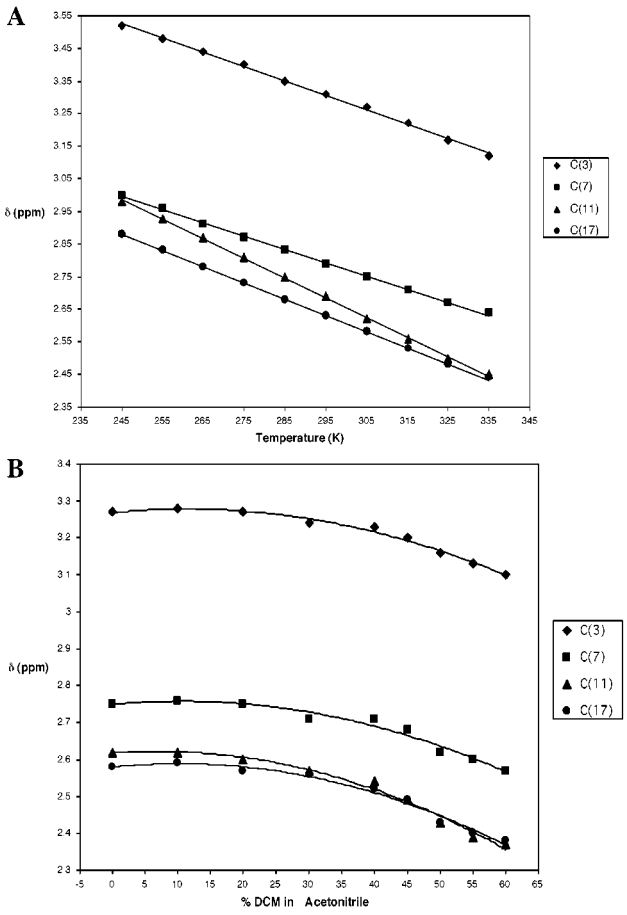

Figure 4. (A) Hydroxyl ^{1}H δ (ppm) as a function of temperature (K). (B) Hydroxyl ^{1}H δ (ppm) as a function of % CD_2Cl_2 in CD_3CN.

hydrogens displayed approximately the same temperature dependence (−0.0045 ppm/K). Furthermore, when CD_2Cl_2 was titrated into an NMR sample composed of CD_3CN (Figure 4B), a similar trend was observed; the three hydroxyl hydrogens [i.e., C(3), C(7), and C(17)] displayed similar dependence, while the C(11) hydroxyl hydrogen revealed a greater solvent dependence. Taken together, these data suggest the C(3), C(7), and C(17) hydroxyl hydrogens may

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participate in intramolecular hydrogen bonds, while the C(11) hydroxyl hydrogen is more solvent exposed. In addition, at low temperature (−8 °C), resolution of the carbamate hydrogens was observed (ca. 0.2 ppm), indicative of NH participation in an intramolecular hydrogen bond. Alternatively, resolution of the carbamate hydrogens could result from temperature-induced slowing of N−C σ bond rotation.[23]

With the NMR and computational results in hand, conformational analysis revealed several structural motifs responsible for the observed helical conformation. The first of these, the *Z*-olefins, are influenced conformationally by A[1,3] strain.[24] This nonbonding interaction stabilizes the C(7−10), C(12−15), and C(20−23) regions of the structure (Figure 5a). Second, polypropionate molecules have been

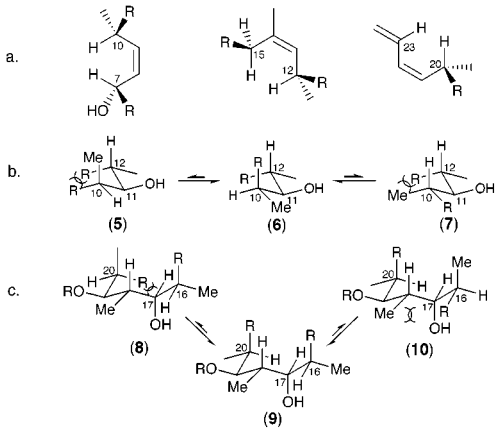

Figure 5. Nonbonded interactions observed in the solution structure of (+)-discodermolide.

shown to adopt conformations that minimize *syn*-pentane interactions.[25] In (+)-discodermolide (**1**) the backbone avoids significant *syn*-pentane interactions at three locations (Figures 5b,c). The first of the *syn*-pentane interactions arises at the C(10−12) unit of the backbone and as such induces the first major turn. The solution conformation of (+)-discodermolide minimizes the C(10−12) *syn*-pentane interactions (e.g., **6**, Figure 5), while other conformers (**5** and **7**) are disfavored due to steric congestion. The second and third *syn*-pentane interactions at C(16−18) and C(18−20), respectively, are responsible for the second major turn in the backbone. Again, the conformation of the natural product in this region (**9**, Figure 5) minimizes the possible *syn*-pentane interactions (**8** and **10**).

Finally, the variable temperature and titration studies suggest that the C(5,6) and C(6,7) bonds may adopt a conformational bias via through-space interaction of the lactone ring oxygen and the C(7) hydroxyl to create a six-membered ring (Figure 3B). Under dynamic conditions, the C(3) and C(17) hydroxyl substituents may interact with the carbamate moiety via long-range (>2.6 Å) hydrogen bonds.[26] Alternatively, rotation about the C(5,6) σ bond places the δ-lactone carbonyl in position to participate in an intramolecular hydrogen bond with the C(19) carbamate (not shown). Such hydrogen bonding would explain the variable temperature experiments and the titration studies.[27] Clearly, the C(11) hydroxyl substituent is significantly more exposed to the surrounding environment, a fact consistent with the variable temperature and titration studies.

In summary, a combination of 1- and 2-D NMR techniques and computational studies reveals that in solution (+)-discodermolide (**1**) exists in a helical conformation remarkably similar to the solid state. Analysis of the solution and solid state conformational properties of the (+)-discodermolide system suggests that A[1,3] and *syn*-pentane nonbonded interactions along the carbon backbone play a major role in defining the overall conformation. Variable temperature and titration NMR experiments also implicated intramolecular hydrogen bonding as a possible conformational influence. Additional studies exploiting the solution and solid state conformation of (+)-discodermolide (**1**) for the design and synthesis of bioactive analogs and for the definition of the bioactive pharmacophore will be reported in due course.

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Supporting Information Available: NMR: 1D ^{1}H (CD_3CN/D_2O); 2D ROESY (CD_3CN); Macromodel 6.0 coordinates for Monte Carlo global minimum. This material is available free of charge via the Internet at http://pubs.acs.org.

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(26) We cannot exclude the possibility that the interactions may occur through bridging water molecules, which is consistent with the anhydrous decomposition of (+)-discodermolide.
(27) A crystal structure of an α,β unsaturated (dehydro) lactone analog of discodermolide has been determined and retains the same conformation as the natural product. Therefore, it is difficult to predict the conformational significance of a long-range intramolecular hydrogen bond between the C(3) OH and the carbamate.