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Tetrahedron Letters 46 (2005) 3677–3682
Tetrahedron Letters

Conformational studies and solution structure of laulimalide and simplified analogues using NMR spectroscopy and molecular modelling

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Received 16 February 2005; accepted 22 March 2005
Available online 8 April 2005

Abstract—The solution structures of the potent microtubule-stabilizing anti-cancer agent laulimalide and simplified analogues were determined by a combination of high field ^{1}H NMR spectroscopic studies (*J*-based configuration analysis and NOESY) and constrained molecular dynamics simulations and discussed in relation to their antiproliferative activity.

The marine macrolide laulimalide (**1**, Fig. 1)[1] represents an attractive lead compound for development as a new structural class of cancer chemotherapeutic agent. Laulimalide stabilizes microtubules in a similar manner to Taxol® (paclitaxel) and inhibits the proliferation of a range of human carcinoma cell lines, including multidrug and paclitaxel resistant cells, at low nanomolar concentrations.[2,3] Moreover, it appears to have a different (and as yet undefined)[4] binding site on tubulin[5,6] and is much less susceptible to P-glycoprotein-mediated multidrug resistance.[3a] This promising biological profile, together with its low natural abundance from its sponge sources, has triggered extensive synthetic efforts, culminating in a number of total syntheses of laulimalide.[2,7,8] Despite these contributions, there is still a supply problem, which renders the development of structurally simplified and, as such, more readily accessible analogues an important goal. For the rational design of such analogues, an understanding of the detailed 3D structure and conformational behaviour of laulimalide should be highly informative.

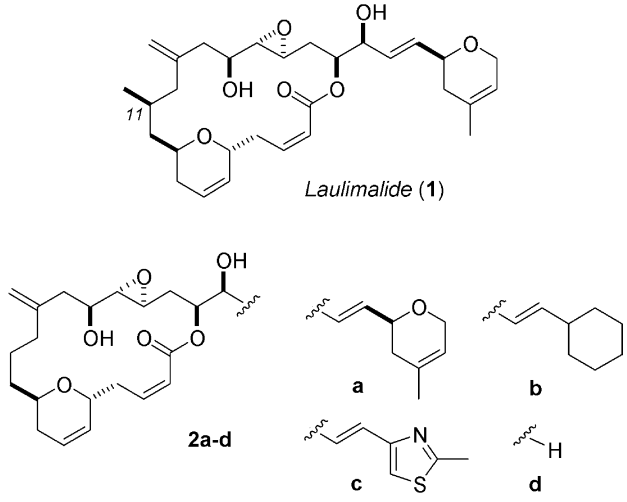

Figure 1. Laulimalide (**1**) and its 11-desmethyl-analogues **2a**–**d**.

While other microtubule-stabilizing agents have been extensively studied by analogue preparation and solution conformational analysis,[9] no such detailed studies have been described for laulimalide and only a limited range of derivatives have been reported to date.[8] So far, only the solid state structure (X-ray crystal structure of laulimalide itself) provides substantive information on the preferred conformation.[1c] Herein, we report the elucidation of the solution structure of laulimalide (**1**), together with selected simplified analogues **2a**–**d** featuring a modified macrocyclic core at C11 and having a

Keywords: Laulimalide; Solution structure; Conformational analysis; Analogues; Anti-cancer agent; Modelling.
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0040-4039/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tetlet.2005.03.149

truncated or replacement side chain at C20,[8g] by ^{1}H NMR spectroscopy and molecular modelling.

The salient structural features of laulimalide include a 20-membered macrolide ring, two dihydropyran units, an epoxide, five double bonds and nine stereogenic centres. As a close mimic of biologically relevant systems, all spectral data for laulimalide were recorded in CD_3OD[10] in a similar manner to our recent conformational studies on dictyostatin, a related microtubule-stabilizing marine macrolide.[9e] Optimum ^{1}H NMR signal dispersion was realized at the highest available field strength (700 MHz), allowing the complete assignment of all resonances.[11] A combination of multiplet analysis,[12] homonuclear decoupling and TOCSY experiments at different mixing times (Fig. 2) was used for the extraction of all $^{3}J_{H,H}$ coupling constants, while information on the spatial relationships between nonadjacent protons was deduced from NOESY experiments (0.5 s mixing time). Based on these data, laulimalide was divided into three subunits—a 'northern' (C13–C19, Fig. 2a) and 'southern' (C1–C12, Fig. 2b) region of the macrocycle, and the side chain (C19–C27, Fig. 2c).[13]

The northern (C13–C19) region (Fig. 2a) is characterized by a sequence of small/large coupling constants and distinctive NOESY data, indicating the relative rigidity within this subunit. In particular, small couplings observed for H-15 to H-16, H-17 to H-18a and H-18a to H-19 suggest *gauche* relationships between these protons. The large couplings for H-15 to H-14a and H-18b to both H-17 and H-19, in combination with a strong NOESY interaction between H-17 and H-19, led to the conformational assignment for the C15–C19 region as shown. Based on this analysis, the epoxide ring is expected to be on the outside of the macrocycle. The predominance of this conformer is further supported by a number of additional NOESY correlations (e.g., H-14b to H-15, H-16 to H-18b).

Conformational assignment of the southern region of laulimalide (Fig. 2b) was based on a combination of NOESY data (H-5 to H-3 and H-4b to H-6) and a large vicinal coupling between H-5 and H-4a. The *gauche* relationship between H-5 and H-4b ($^{3}J_{H,H} = 2.9$ Hz) supports this assignment. A strong NOESY correlation from H-3 to H-5, together with a large dipolar coupling

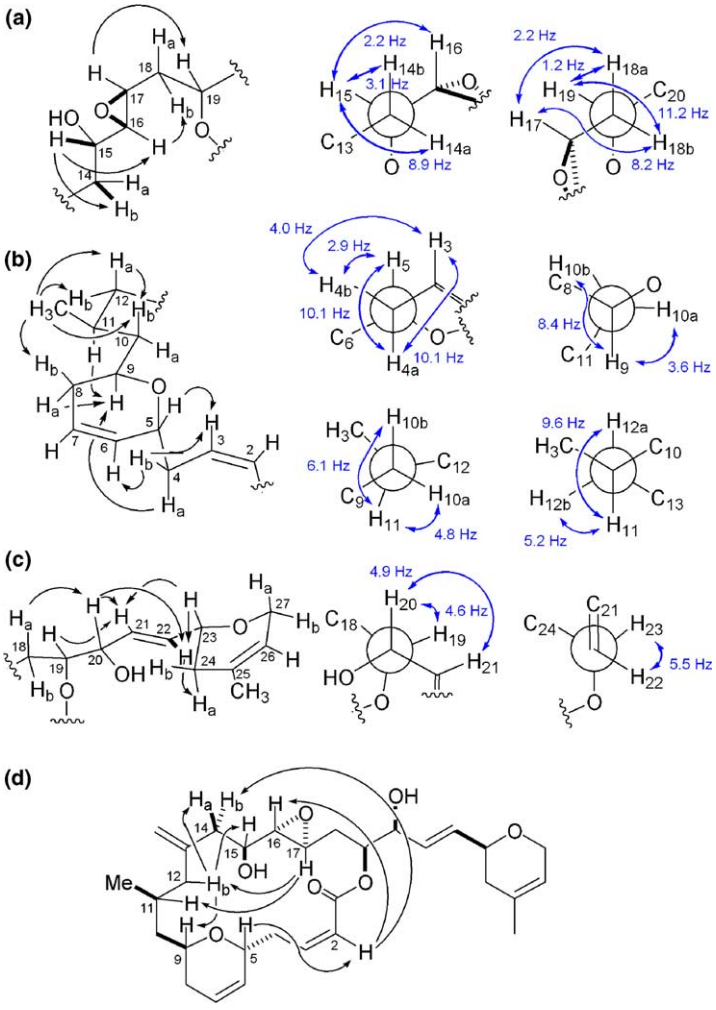

Figure 2. Laulimalide: selected NOESY correlations (single arrows) and $^{3}J_{H,H}$ values (double arrows) within (a) the C14–C19 region; (b) the C2–C12 region and (c) the C18–C27 region; (d) transannular NOESY correlations.