Pergamon

Tetrahedron Letters, Vol. 37, No. 2, pp. 159-162, 1996

0040-4039/96 $15.00 + 0.00

0040-4039(95)02113-2

Structures and Absolute Configurations of the Marine Toxins, Latrunculin A and Laulimalide

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Key words: Macrolide, Fijianolide, 2-Thiazolidinone, Dihydropyran, X-ray.

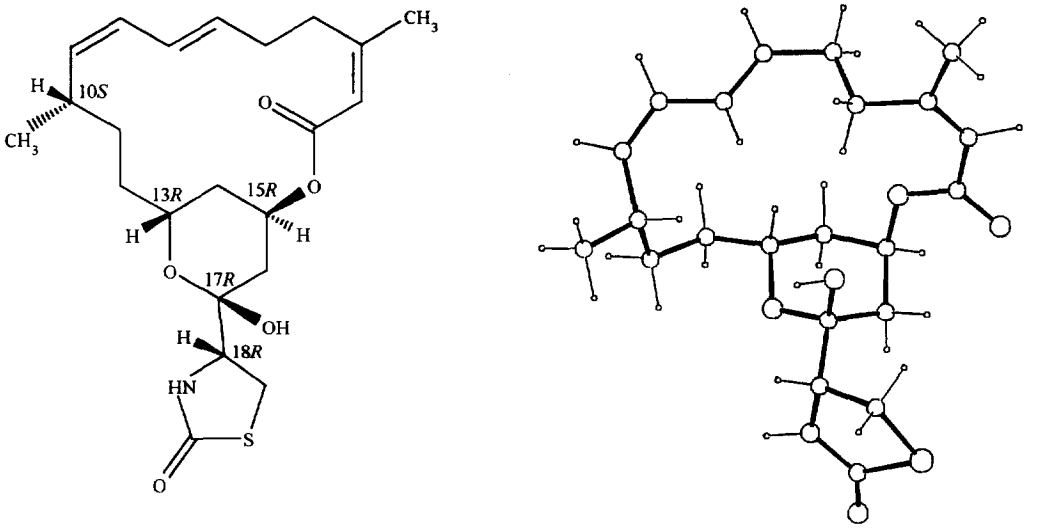
Abstract: Latrunculin A (1) and laulimalide (2) were isolated from Fasciospongia rimosa collected in Okinawan waters. By purification, single crystals were obtained thereby enabling the absolute configurations of 1 and 2 to be determined by X-ray analysis.

Over the past few years macrolides of marine origin have continued to be of interest on account of their diverse biological properties. Perhaps the best known is bryostatin 1 which is being evaluated as an anti-tumor agent.[1,2] Two other pertinent examples are latrunculin A (**1**) and laulimalide (**2**). The former was first isolated from the Red Sea sponge *Latrunculia magnifica*.[3] Later, it was found together with **2**, also known as fijianolide B,[4] in three Pacific sponges, *Spongia mycofijiensis*,[4] *Hyattela* sp.,[5] and an unidentified specimen.[6] It was noticed that the liquid exudate obtained by squeezing *L. magnifica* was extremely toxic to fish. Apart from this primary effect to protect the sponge against predators, experiments showed that **1** exerts a strong reversible effect on microfilament organization in cultured mouse neuroblastoma cells[7] and **2** displays potent cytotoxicity against the KB cell line.[5] Unfortunately, owing to the nature of the original samples; **1** was a foam and **2** a viscous oil; their structures could only be partially determined. The relative configuration of latrunculin A was extrapolated from that of its methyl acetal derivative, the structure of which was secured by X-ray, although the accurate location of the hydrogen atoms proved difficult.[3] Subsequently, its absolute configuration was designated as 10S, 13R, 15R, 17R and 18R on the basis of its degradation to ethyl 2-oxo-4-thiazolidinecarboxylate which was identical to a sample prepared from L-cysteine.[8]

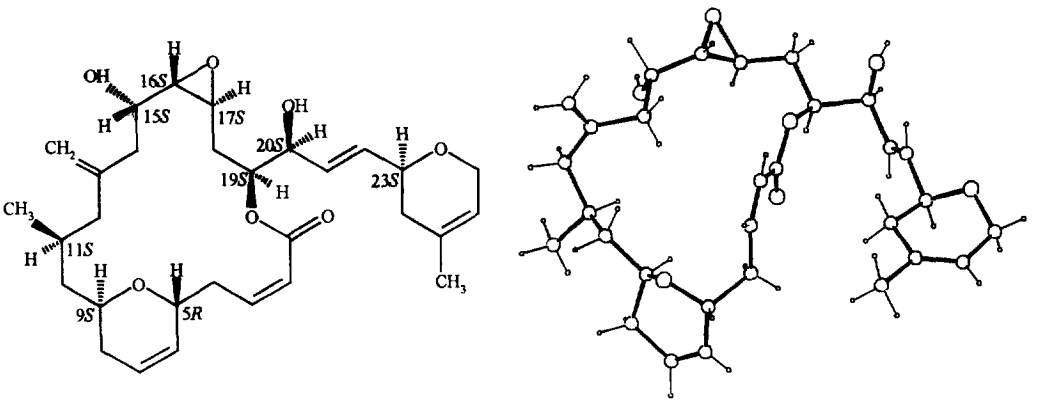
In the case of laulimalide, 2D-NMR spectroscopy enabled only the overall skeleton and the relative stereochemistry of the epoxide-lactone portion to be elicited. Although the two studies[4,5] were essentially in

agreement, there was some ambiguity about the conformations of the substituents on the dihydropyran ring (v. infra). Clearly, in the light of the pharmaceutical potential of these entities, the confirmation of the structure of **1** and the elucidation of that of **2** are long overdue. We now describe the re-discovery of **1** and **2**, their characterization as optically pure compounds and the unequivocal determination of their absolute configurations.

A sample (4.48 kg) of the sponge *Fasciospongia rimosa*[9] was collected from underwater caves on Shimoji island, Okinawa, in November 1993. Extraction with acetone (4 x 6 L), concentration of the extracts,



Latrunculin A (**1**)



Laulimalide (**2**)

and further extraction of the resulting aqueous suspension with ethyl acetate afforded an oil (39.3 g). Vacuum flash chromatography of the oil over silica gel by elution with step gradients of hexane/AcOEt/MeOH gave a fraction (7.68 g) which by preparative HPLC (Novapak, silica, hexane/AcOEt, 1:3) finally furnished **1** (5.94 g) and **2** (930 mg). Single crystals of **1** and **2** suitable for X-ray analysis were grown from solutions in CH_2Cl_2/AcOEt and aqueous methanol respectively.[10,11]

The 16-membered macrolide structure previously proposed for latrunculin A (**1**) was confirmed and its absolute configuration confirmed as 10S, 13R, 15R, 17R, 18R (Fig. 1). The tetrahydropyran ring exists as a chair conformation in which the hydroxyl group at the potentially epimerizable C17 position retains its *syn*-axial relation with respect to the C15-carboxyl substituent. This stereochemical preference is probably dictated by the anomeric effect of the pyran oxygen atom as well as by the bulk of the 2-thiazolidinone substituent. The ring of the latter is bent into an envelope conformation so that the C19 atom is pushed out of the plane ($\Delta Cs = 0.015(4)$).[12]

The absolute configuration of laulimalide (**2**) was determined as 5R, 9S, 11S, 15S, 16S, 17S, 19S, 20S, 23S (Fig. 2). Thus, the gross structural elements previously deduced were found to be correct. The macrolide ring consists of 18 atoms within the inner perimeter and encompasses *trans*-substituted epoxide and dihydropyran rings. Both the intracyclic dihydropyran and its external companion adopt half-chair conformations. The X-ray analysis also permits a resolution of the contradictory conformational assignments made by NMR spectroscopy for the hydrogen atoms attached at the C5 and C9 positions on the 6-membered ring. It is now seen that C5-H is quasi-equatorial and C9-H axial.

The present results not only provide indispensable information on the molecular geometry of these unusual 18- and 20-membered lactones, but also reveal conformational preferences, at least those operating in the solid state. Finally, it is worth noting that, unlike bryostatin 1 and miyakolide, the macrolide ring in **1** and **2** is attached to the pyran ring in *trans*-fashion. It remains to be seen how these structural differences and ring-size will have a bearing on biological activities. Such studies are in progress and the results will be reported elsewhere.

Acknowledgments. We thank PharmaMar S.A., Tres Cantos, Spain, for partial support of this research.

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9. Taxonomic identification was performed by Dr. J.N.A. Hooper, Queensland Museum, Brisbane, Qld., Australia. A voucher specimen (No. G301467) has been deposited at the museum.
10. Latrunculin A was obtained as colorless crystals, m.p.110-112°, $[\alpha]_D^{20}$ +240 (c 0.19, $CHCl_3$). IR (CCl_4): 3430, 3300, 2960, 1690, 1270, 1090, and 1065 cm^{-1}. The ^1H- and ^{13}C-NMR spectra in $CDCl_3$ were identical to those reported elsewhere (see ref. 3). Laulimalide afforded colorless crystals, m.p. 116-119°, $[\alpha]_D^{29}$ -200 (c 1.03, $CHCl_3$). ^1H-NMR ($CDCl_3$): δ 0.82 (d, J = 6.4 Hz, 3H, H30), 1.33 (ddd, J = 3.5, 4.9. 14.3 Hz, 1H, H10a), 1.45 (ddd, J = 7.6, 7.6, 14.3 Hz, 1H, H10b), 1.49 (ddd, J = 9.5, 11.3, 14.4 Hz, 2H, H18a), 1.69 (s, 3H, H28), 1.72 (m, 1H, H11), 1.78 (dd, J = 10.1, 12.8 Hz, 1H, H12a), 1.87 (brd, J = 17.1 Hz, 1H, H24a), 1.93 (m, 1H, H8a), 2.00 (dd, J = 8.9, 15.7 Hz, 1H, H14a), 2.02 (m, 2H, H8b,H24b), 2.12 (brd, J = 15.7 Hz, 1H, H14b), 2.22 (ddd, J = 4.0, 6.4, 16.8 Hz, 1H, H4a), 2.37 (m, 1H, H18b), 2.38 (m, 1H, H12b), 2.90 (t, J = 2.6 Hz, 1H, H16), 3.08 (ddd, J = 2.5, 3.7, 9.1 Hz, 1H, H17), 3.72 (ddd, J = 1.2, 10.1, 16.8 Hz, 1H,H4b), 3.76 (m, 1H, H9), 4.03 (m, 1H, H23), 4.07 (m, 1H, H15), 4.17 (m, 2H, H27), 4.22 (q, J = 5.5 Hz, 1H, H20), 4.31 (brd, J = 9.5 Hz, 1H, H5), 4.85 (brs, 1H, H29a), 4.86 (brs, 1H, H29b), 5.15 (ddd, J = 1.5, 5.2, 11.3 Hz, 1H, H19), 5.42 (brs, 1H, H26), 5.69 (brd, J = 10.3Hz, 1H, H6), 5.75 (ddd, J = 1.2, 5.8, 15.6 Hz, 1H, H21), 5.83 (m, 1H, H7), 5.87 (ddd, J = 1.2, 5.2, 15.6 Hz, 1H, H22), 5.91 ddd, J = 1.2, 2.4, 11.6 Hz, 1H, H2), 6.44 (ddd, J = 3.7, 10.1, 11.6 Hz, 1H, H3). ^{13}C-NMR ($CDCl_3$): δ 20.7 (C30), 22.9 (C28), 29.5 (C11), 31.6 (C8), 33.3 (C18), 33.8 (C4), 35.6 (C24), 37.1 (C14), 43.4 (C10), 45.5 (C12), 52.1 (17), 60.7 (C16), 65.6 (C27), 66.5 (C15), 67.9 (C9), 72.3 (C19), 73.1 (C5), 73.2 (C20), 73.4 (C23), 112.5 (C29), 119.7 (C26), 120.5 (C2), 125.2 (C7), 128.5 (C6), 128.7 (C21) , 131.2 (C25), 133.8 (C22), 144.9 (C13), 150.3 (C3), 166.0 (C1).
11. Both compounds crystallized in the orthorhombic system, space group $P2_12_12_1$. Data were collected at room temperature for 1 and 170K for 2 on an automatic four-circle Nonius CAD-4 diffractometer with monochromated MoKα (1) and CuKα (2) radiation. Both structures were solved by direct methods and refined by full matrix least-squares analysis. All the coordinates of the hydrogen atoms were observed and refined. The chirality of the structures was refined and the absolute structure parameter[13] converged to x = -0.07(23) for 1 and 0.15(43) for 2. It should be noted that the large values of the estimated standard deviations associated with the x parameters are due to the low anomalous contributions of the constituent atoms. In both compounds, the molecular packing is fixed by hydrogen bonds involving the hydroxyl groups (1: O(4)....O(5)1/2+x,1/2-y, 1-z = 2.770(8) Å; 2: O(4)....O(5)1/2+x, 3/2-y, 1-z = 2.920(6); O(6)....O(7) 1/2+x, 1/2-y, 1-z = 2.796(6) Å. The correct choice of solvent was particularly crucial for latrunculin A (1) since its successful crystallization depended on the incorporation of a molecule of AcOEt into the crystal lattice. Although the atomic displacement parameters of the guest molecule are large, no atomic disorder was observed. *Crystal data:* 1, $C_{22}H_{31}NO_5S$ / $C_4H_8O_2$, m = 509.7; $P2_12_12_1$, a = 9.535(2), b = 11.683(1), c = 25.259(7) Å, Z = 4, Dc = 1.20 gr·cm^{-3}, μ = 0.157 mm^{-1}, F000 = 1096. R = 0.061, ωR = 0.033 ($\omega = 1/\sigma^2(Fo)$) for 2741 observed reflections ($|Fo| > 4\sigma(Fo)$). 2, $C_{30}H_{42}O_7$, m = 514.7, $P2_12_12_1$, a = 5.6664(3), b = 14.821(1), c = 33.723(3) Å, Z = 4, Dc = 1.21 gr·cm^{-3}, μ = 0.650 mm^{-1}, F000 = 1112. R = 0.046, ωR = 0.036 ($\omega = 1/\sigma^2(Fo)$) for 37861 observed reflections ($|Fo| > 4\sigma(Fo)$). Crystallographic data have been deposited with the Cambridge Crystallographic Data Center, University Chemical Laboratory, 12 Union Road, Cambridge CB2 1EZ, England.
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(*Received in Germany* 11 *September* 1995; *accepted* 6 *November* 1995)