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LATRUNCULIN, A NEW 2-THIAZOLIDINONE MACROLIDE FROM THE MARINE SPONGE LATRUNCULIA MAGNIFICA

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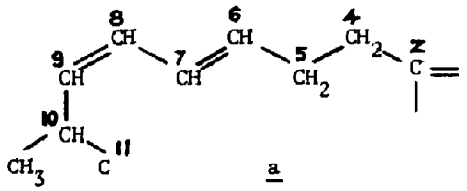
Abstract. Three new toxins were isolated from the Red Sea sponge Latrunculia magnifica. A full structure determination of these toxins, by spectroscopic methods, was aided by an X-ray diffraction analysis of a crystalline derivative (1b) of latrunculin-A. The Latrunculins exhibit a new class of 14 and 16 membered macrolides to which the rare 2-thiazolidinone moiety is attached. A biogenesis of these biologically active compounds is put forward.

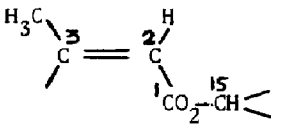
Among the most prominent sponges in the Gulf of Eilat (the Red Sea) are colonies of the branching red-coloured Latrunculia magnifica Keller, occuring at a depth of 6.0 to 30.0 m, and clearly observable under water from relatively long distances. As reported by us previously[1], colonies of this sponge were never observed to be damaged or eaten by fishes. Furthermore, when squeezed manually these sponges exude a reddish fluid, a "juice" which causes fish to escape immediately from the sponge vicinity. When L. magnifica is squeezed into an aquarium it causes poisoning and death of the fish within 4-6 minutes[2]. In our previous report[1] we have described the isolation of the toxin from the sponge and several of its biological activities on fish, cholinesterase and inhibition of microorganisms' growth. As is well documented with many marine organisms, their chemical content changes with various parameters which are as yet not well understood. Similar to that is the case of the L. magnifica. The choice of the specimen with which we had started in 1975, turned out to be far from the best.

The toxic fraction in this collection did not contain a single toxin but rather, as we now know, two closely related isomers, contaminated by glycerides (which could not be easily removed by silica gel chromatography). Collections performed during the years 1976-1979 supplied us with at least three different Latrunculia species, or specimens, as far as the toxin content is concerned[3]. A combination of Sephadex LH-20 and Silica-gel chromatographies enabled us to get rid of the accompanying glycerides, and obtain three pure toxins which were named Latrunculin-A (1a), Latrunculin-B (2) and Latrunculin-C (3). It was, however, not before we succeeded in preparing a crystalline derivative of 1a that we could determine unequivocally the full structure of these toxins. The latrunculins turned out to be most interesting new macrolides to which the rare 2-thiazolidinone moiety is attached.

Latrunculin-A (1a) is an oil, $[\alpha]_D^{24^\circ}$ + 152° (c=1.2, $CHCl_3$), λ_{max}(MeOH)218(23500),268(sh). The molecular weight and formula $C_{22}H_{31}NO_5S$ were established by mass spectroscopy. The 270MHz ^{1}H-NMR spectrum together with the 22.63MHz ^{13}C-NMR spectrum[4] allowed the definition of the following structural units:



a



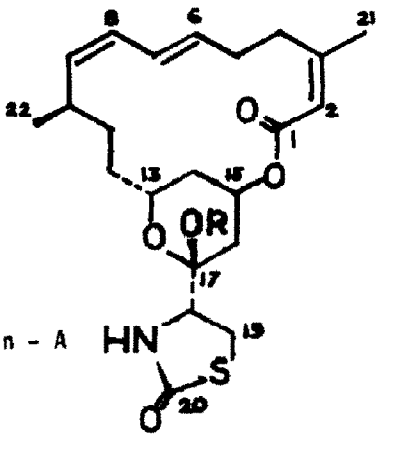
b

Extensive decoupling studies and chemical shift values suggested the above partial structural units. Moieties a and b could be linked to each other following a microozonolysis experiemnt which produces levulinaldehyde, indicating that group Z in moiety a has to be carbon No.3 (moiety b). According to the elemental composition of 1a (8 unsaturations) and the ^{13}C-NMR spectrum, compound 1a contains three double bonds[4] and two carbonyls (δ 175.5s and δ 166.0s). Thus, latrunculin-A was expected to be tricyclic.In addition to the diene and the αβ-unsaturat ester embodied in moieties a and b, compound 1a was believed to possess an amide (IR ν_{max} 3430,3350br and 1690 cm^{-1})[5]; ^{13}C-NMR[4] and ^{1}H-NMR δ 5.80-6.30 (1H, exchangeable after longer time). A singlet at δ 96.9 in the ^{13}C-NMR spectrum of 1a suggested a ketal type carbon as th site bearing the two remaining oxygens of the molecule. Latrunculin-A could not be acetylate under mild conditions (Ac_2O/Pyr. r.t.), however, water could be eliminated to give compound 4 The ^{13}C-NMR spectrum of the two newly formed double bond carbon atoms (δ 156.2s and δ96.7d) w indicative of an enolether of type $\geq$CH-O-$\overset{13}{\underset{|}{C}}$=$\overset{17}{C}$H-^{16} thereby confirming the existence of a hemiketa in 1a. Indications on the N and S containing moiety were obtained from the mass spectrum. M significant in this spectrum of compound 1a (and other closely related compounds in the group were the m/e 102 (10%) and m/e 301 [M^+-H_2O-102] (100%) peaks (14eV). HR-MS determined the co position of these peaks to be C_3H_4NOS(m/e 102) and M-H_2O-C_3H_4 NOS (m/e 301). The structure of the latter C_3H_4NOS group was clarified unequivocally only after performing an x-ray diffracti analysis according to which it turned out to be the 2-thiazolidinone heterocycle. The x-ray diffraction analysis was performed on methylated latrunculin-A, i.e. on compound 1b[7], in whic the hemiketal was transformed into a ketal (this was the only crystaline compound among the hitherto synthesized derivatives of 1a). In spite of the relative complexity of the crystal structure of 1b (vide infra), it was preferred over Latrunculin-B[8], and was chosen for the crystallographic investigation. Its crystals are tetragonal, space group $P4_122$, with unit ce dimensions: a=11.396 (1)Å and c=36.735 (3)Å. The density of the crystals, measured by the flotation method is 1.19g$\cdot$ cm^{-3} and that calculated for eight molecules of $C_{23}H_{33}NO_5S$ in the cell is 1.21g$\cdot$cm^{-3}. Intensity data were collected on a CAD 4 automatic diffractometer, using Ni-filtered Cu radiation, by the θ-2θ scan technique. The maximum scanning time of a reflect was fixed at 2 minutes, the prescan speed being 4°/minute . 2465 unique reflections, with po itive I, were obtained from the angular range: $2\leq\theta\leq70°$. Out of these, 1417 reflections with $I>3\sigma$ (I) were used in the structure determination and refinement. The structure was solved b direct methods and ΔF syntheses and was conventionally refined to R=0.084 with the aid of a local least squares program. Full-matrix anisotropic refinement of the parameters of the 30 H atoms was carried out. The drawing of 1b projected approximately onto the best plane passi through all the atoms is shown in Fig.1.

The bond distances and angles are in good agreement with those reported in the literatu Except from the somewhat short intermolecular distance $N_1\ldots O_5$=2.95Å (which may be a weak H b there are no unacceptably short contacts in the structure.

As anticipated from the ^{1}H-NMR data (δ-values and coupling constants) the geometry of th double bonds is 2,3Z, 6,7E and 8,9Z. Furthermore, H-13 and H-15 were found to be axial and equatorial respectively in the solid state. The latter conclusion is consistent with the res of the ^{1}H-NMR study[7], thus indicating that the conformation of the tetrahydropyrane is the sa in both liquid and solid states. This observation is of particular importance for the C-17

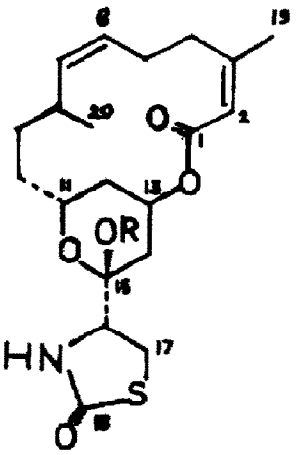
figuration assignment in compound 1a. Ketalization of the hemiketal of 1a is expected to go through the oxonium ion and thus C-17 loses its original configuration. However, as the 2-thiazolidinone group of compound 1b in both solid and liquid states is equatorial[9], and as the H-13 and H-15 multiplicities in 1a[4] are essentially the same as in 1b[7], the configuration of C-17 is also expected to be the same in 1a as in 1b.



Latrunculin - A

1a R=H

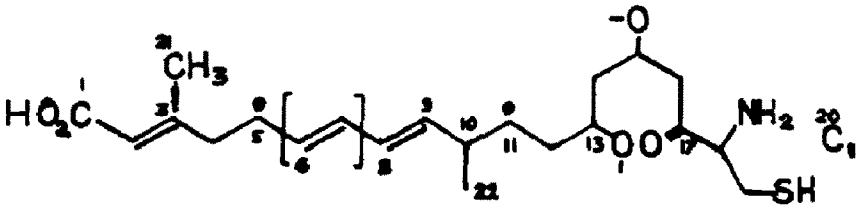
1b R=CH_3



Latrunculin - B

2 R=H

According to the various spectroscopic data, latrunculin-B (2)[10] is a 14-macrolide which differs from the 16-macrolide 1a only in having a monoene rather than a diene moiety. On the other hand, latrunculin-C is a stereoisomer of 1a[11]. To the best of our knowledge, the latrunculins are the first marine macrolides and, moreover, the first natural products containing a 2-thiazolidinone terminus. Finally, the following biogenesis of the latrunculins is suggested, where the compounds are polyketides starting with cysteine as the first acyl group:



We suggest that the original oxygens are reduced at C-5 and 11(•) and eliminated from C-3,(7) and 9; C-20 and 21 are two extra C_1-units while C-22 (together with C-9 & 10) may originate from a propionate.

Acknowledgements: We wish to express our appreciation to Dr. Y. Loya and coworkers for collecting the sponge and to Professor Vacelet for the identification.

References and Notes

1. I. Néeman, L. Fishelson and Y. Kashman, Marine Biology 30, 293 (1975).

2. The toxin causes excitation of the fish in seconds followed by haemorrhage, losing of balance and finally, after a few minutes, death.

3. Professor J. Vacelet, who has identified the L. magnifica sponge, brought to our attention that there is another species of Latrunculia in the Red Sea, which is perhaps a synonym, namely, L. corticata Carter. This matter has to be further explored.

4. m/e(14eV.%)421(M^+,$C_{22}H_{31}NO_5S$,20), 403(M^+-H_2O,47), 385(46), 301(100), 149(48), 135(50), 102(and 85(44); ^{1}H-NMR(Bruker 270MHz,$CDCl_3$,δ): 6.41dd(J=15,10.5Hz,H-7), 5.98t(J=10.5,H-8), 6.3 5.8(NH), 5.74dt(J=15,4.5,H-6), 5.69d(J=1.3,H-2), 5.43bt(J=3,H-15), 5.02t(J=10.5,H-9), 4.29m

(H-13),4.10-3.90(OH),3.87dd(J=8,7,H-18),3.51dd(J=11.5,7,H-19),3.48dd(J=11.5,8,H-19'),3.00m 2.83m(H-10),2.60m(H-4'),2.26m(H-5,5'),1.92d(J=1.3,Me(21)),0.98d(J=6.7,(Me(22);^{13}C-NMR(Brul 22,63MHz $CDCl_3$,δ): 175.5s,166.0s,158.3s,136.5d,131.8d,127.3d,126.3d,117.6d,96.9s,68.1d,62. 62.1d,35.1t,32.7t,32.1t,31.8t,31.2t,30.6t,29.2d,28.7t,24.7q,21.8q.

5. Hydrogenation of the unsaturated lactone splits into two the 1690 absorption (1690,1730cm
6. Elimination of 1a ($SOCl_2$,Pyr.0°,20min) gave compound 4 as an oil, ν_{max}3390,2890,1670,1380 990cm^{-1}; m/e(12eV): 403($M^+C_{22}H_{29}NO_4S$): ^{1}H-NMR: 5.22bd(J=5Hz,H-16) and 5.19bt (J=5,H-15).
7. 1b,m.p. 164°-165°(benzene); $[\alpha]_D^{24}$+315°(c=0.33,$CHCl_3$); λ_{max}(MeOH)218(25,800) and 269(sh); $\lambda_{max}^{CCl_4}$ 3420,3200,1690 & 1680cm^{-1}; m/e(70eV,%): 403(M^+-MeOH,52),385(43),333(M^+-C_5H_4NOS,66),1 (100) and 102(C_5H_4NOS,12);^{1}H-NMR($CDCl_3$,δ): 5.17bt(H-15),4.15m(H-13) and 3.33s(OCH_3);^{13}C-NM ($CDCl_3$,δ): 99.9s(C-17),56.7d(C-18) and 47.9q(OMe).
8. Latrunculin-B (2) could indeed be crystallized but x-ray work was not undertaken due to very poorly diffracting crystals.
9. The 2-thiazolidinone, the largest tetrahydropyrane substituent, is expected to be equatori
10. $[\alpha]_D^{24}$ +112°(c=0.48,$CHCl_3$); λ_{max}(MeOH): 212nm(17200) and 269(sh); $\nu_{max}^{CHCl_3}$ 3520,3400,1675cm^{-1} m/e(14eV,%): 377(M^+-H_2O,50),275(M^+-H_2O-C_5H_4NOS,40),149(52),81(87) and 57(100);^{1}H-NMR(CDCl$_3$ 6.10-5.90(NH),5.67d(J=1.5,H-2),5.43bt(J=3,H-13),5.25dt(J=11.2,3.3,H-6),5.04t(J=11.2,H-7), 4.24bt(J=10,H-11),3.95-3.85(OH),3.83dd(J=8.6,6.3,H-16),3.47dd(J=11.5,8.6,H-19),3.39dd(J=11 6.3,H-19'),1.90d(J=1.5,Me(19)),0.95d(J=6.6,Me(20)); ^{13}C-NMR($CDCl_3$,δ): 175.3s,165.6s,154.7s 135.9d,127.6d,118.0d,97.7s,68.7d,62.6d,61.8d,35.8t,35.4t,31.8,31.2,31.2,28.9,28.7,26.9,24. and 22.3q.
11. There are only minor ^{1}H-and ^{13}C-NMR differences between latrunculin-A and -C, e.g. δ(Me(2 in case of 3, is 0.95d.

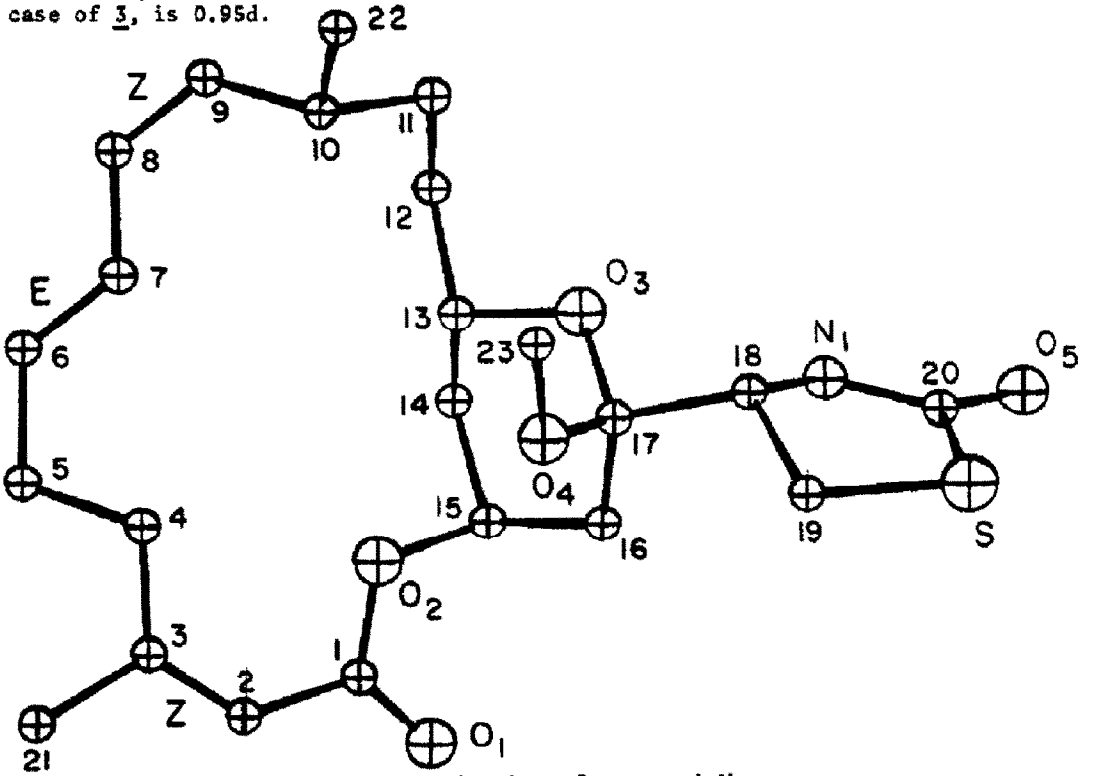


Fig. 1. ORTEP drawing of compound 1b.

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