New Cytotoxic Macrolides from the Sponge *Fasciospongia rimosa*

Jun-ichi Tanaka, Tatsuo Higa,* Gérald Bernardinelli,† and Charles W. Jefford*†
Department of Marine Sciences, University of the Ryukyus, Nishihara, Okinawa 903-01
†*Department of Organic Chemistry and Laboratory of Crystallography, University of Geneva, 1211 Geneva 4, Switzerland*

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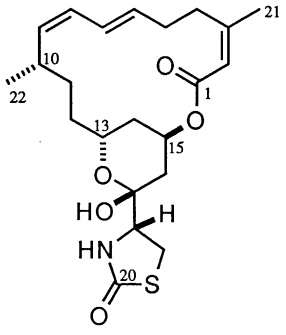
Two new cytotoxic macrolides, latrunculin S and neolaulimalide, have been isolated from the sponge *Fasciospongia rimosa*, and their structures determined by NMR and chemical correlation with known congeners.

In our quest for antitumor agents from marine sources, we recently isolated two known macrolides, latrunculin A (**1**) and laulimalide (**2**) as major cytotoxic constituents of an Okinawan sponge designated as *Fasciospongia rimosa*.[1] The same combination of these macrolides of different classes has previously been reported to occur in Pacific sponges and nudibranchs.[2-4] Since we obtained **1** and **2** in crystalline form for the first time, we were able to examine their crystal structure by X-ray[5] and confirmed the absolute configuration of **1**, which had previously been obtained indirectly by X-ray of a derivative and chemical degradation.[6] We also determined the absolute configuration of laulimalide (**2**, also known as fijianolide B), for which a gross structure with only partial stereochemistry had been previously proposed.

In view of the biological significance[2,3,7] of these unique macrolides we further examined the constituents of the sponge and have isolated two new related compounds, latrunculin S (**3**) and neolaulimalide (**4**) as minor cytotoxic constituents. In this paper we report the isolation and structure elucidation of these new macrolides.

The ethyl acetate soluble oil (39.3 g) obtained from an acetone extract of *F. rimosa* (4.48 kg) was separated by vacuum flash chromatography on silica gel into eight fractions. Further separation of the middle fractions furnished most of the major components **1** and **2** as described earlier.[5] Repeated HPLC separation of the 7th fraction gave **3** (7 mg) and **4** (8 mg), both as glassy materials together with the known isolaulimalide (**5**, 127 mg).



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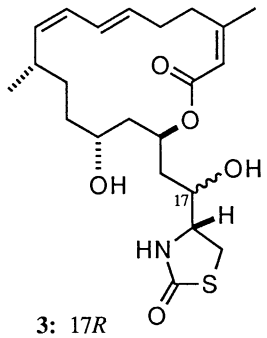
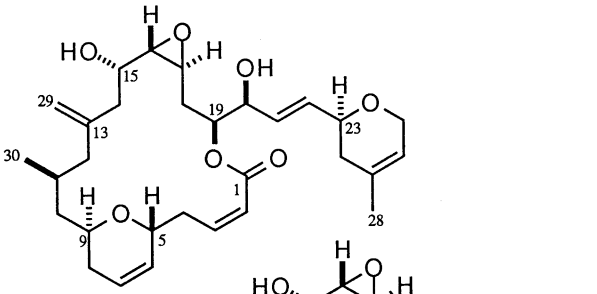
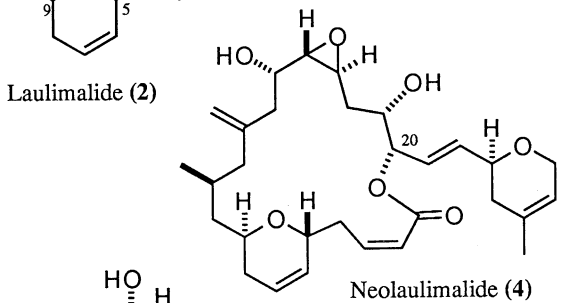


3: 17*R*
6: 17*S*

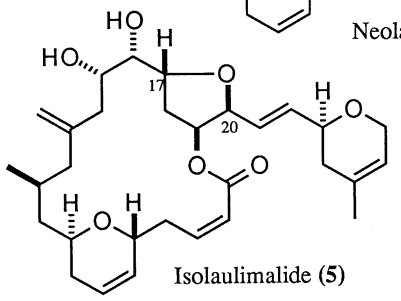
Latrunculin S (**3**), $[\alpha]_D^{26}$ +110° (c 0.187, $CHCl_3$), $C_{22}H_{23}NO_5S$ (*m/z* 423.2083, Δ 0.5 mmu) showed ^1H and ^{13}C NMR data[8] similar to those of **1**. The ^{13}C NMR signal at δ 97.1 s for the hemiacetal carbon in **1** was replaced by a signal at δ 69.6 d (oxymethine carbon) in **3**, suggesting that **3** was a dihydro derivative of **1** in which the tetrahydropyran ring had opened. The overall structure of **3** was secured by 2D NMR experiments (COSY, HMQC, HMBC). Structural and stereochemical correlation of **3** with **1** was provided by $NaBH_4$ reduction of **1**. When **1** was treated with $NaBH_4$ in MeOH, two diastereomeric alcohols (**3**, **6**)[9] were obtained in 52 and 42 % yield, respectively. The major product was shown to be identical to **3** by optical rotation and ^1H and ^{13}C NMR spectra. In order to determine the absolute configuration at C-17 of **3**, it was treated with (*S*)- and (*R*)-MTPA acid by modified Mosher's method.[10] Fortunately, the MTPA esters[11] formed preferentially with the 17-hydroxy group. The Δδ (δ**3**-*S*-MTPA - δ**3**-*R*-MTPA) values for H-15 (+0.10), H-16a (+0.09), H-16b (+0.11), H-18 (-0.12), H-19a (-0.15), and H-19b (-0.12) clearly indicated the 17*R* configuration. Thus, the absolute configuration of **3** was confirmed as 10*S*, 13*R*, 15*R*, 17*R*, 18*R*.



Laulimalide (**2**)



Neolaulimalide (**4**)



Isolaulimalide (**5**)

Neolaulimalide (**4**), $[\alpha]_D^{26}$ -57° (c 0.087, $CHCl_3$) had the same molecular formula $C_{30}H_{42}O_7$ (*m/z* 515.3008, Δ -0.1 mmu) (deduced from HR FABMS) as that of **2**. The ^1H and ^{13}C NMR spectra[12] were closely related to those of **2**, suggesting overall similarity of structure. The gross structure was elucidated by

analysis of the 2D NMR (COSY, TOCSY, HMBC) data. The connectivity studies revealed the presence of a hydroxyl group at C-19 (δH 3.94 m, δC 70.6 d) and an acyloxy function at C-20 (δH 5.32 dd, δC 77.0 d), suggesting the expansion of the lactonic ring size to 21 in **4** from the 20-membered ring in **2**. As shown by previous workers,[2] laulimalide (**2**) could be easily isomerized to isolaulimalide (**5**) by acid treatment.[13] When **4** was similarly treated with CSA in $CDCl_3$, the reaction was much slower (complete in about 48 h), and the product obtained was identical with **5** in optical rotation and ^1H NMR spectrum. Thus, neolaulimalide (**4**) has the same stereostructure and absolute configuration as those of **2** and **5**.

The cytotoxicity of latrunculin S (**3**) against P388, A549, HT29, and MEL28 cell lines was in the range IC_{50} 0.5-1.2 μg/mL, while that of neolaulimalide was 0.01-0.05 μg/mL. The latter value was the same as that observed for laulimalide (**2**) in the same assay.

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References and Notes

1 Taxonomic identification was performed by Dr. J. N. A. Hooper, Queensland Museum, South Brisbane, Queensland, Australia. A voucher specimen (No. G301467) has been deposited at the museum.

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8 **3:** IR ($CHCl_3$) 3540, 1695, 1680, 1600 cm^{-1}; ^1H NMR ($CDCl_3$) δ 6.50 (1H, brs, NH), 6.17 (1H, dd, *J* = 10.7, 14.7 Hz, H7), 5.94 (1H, t, *J* = 10.7 Hz, H8), 5.72 (1H, ddd, *J* = 5.7, 8.2, 15.3 Hz, H6), 5.68 (1H, s, H2), 5.03 (1H, m, H15), 4.98 (1H, t, *J* = 10.7 Hz, H9), 3.89 (1H, m, H13), 3.85 (2H, m, H17,18), 3.49 (1H, dd, *J* = 7.3, 11.3 Hz, H19b), 3.43 (1H, dd, *J* = 5.2, 11.3 Hz, H19a), 3.12 (1H, ddd, *J* = 5.5, 8.2, 12.8 Hz, H4b), 2.56 (1H, m, H10), 2.50 (1H, m, H4a), 2.34 (1H, m, H5b), 2.14 (1H, m, H5a), 2.09 (1H, m, H16b), 1.91 (3H, s, H21), 1.87 (2H, m, H14), 1.85 (1H, m, H16a), 1.57 (1H, m, H11b), 1.45 (1H, m, H12b), 1.33 (1H, m, H12a), 1.22 (1H, m, H11a), 0.99 (3H, d, *J* = 6.7 Hz, H22); ^{13}C NMR ($CDCl_3$) δ 175.4 s, 166.7 s, 159.3 s, 135.9 d, 133.4 d, 128.1 d, 125.8 d, 117.0 d, 69.6 d, 69.2 d, 66.9 d, 58.7 d, 41.2 t, 36.8 t, 34.1 t, 32.4 t, 32.0 t, 30.8 t, 30.5 d, 29.6 t, 24.7 q, 21.6 q; EIMS *m/z* 423 (M^+, 22), 405 (8), 107 (85), 79 (100 rel %).

9 **6:** ^1H NMR ($CDCl_3$) δ 6.07 (1H, dd, *J* = 10.4, 15.0 Hz), 5.93 (1H, t, *J* = 10.4 Hz), 5.72 (1H, m), 5.72 (1H, s), 5.06 (1H, m, H15), 4.97 (1H, t, *J* = 10.4 Hz), 3.74 (2H, m, H13,18), 3.42 (1H, ddd, *J* = 2.0, 8.0, 10.4 Hz, H17), 3.36 (1H, ddd, *J* = 3.7, 9.8, 12.2 Hz), 3.31 (1H, dd, *J* = 7.3, 11.0 Hz, H19b), 3.13 (1H, dd, *J* = 8.0, 11.0 Hz, H19a), 1.94 (3H, s), 1.91 (1H, m, H14b), 1.74 (1H, ddd, *J* = 3.7, 8.9, 12.2 Hz, H14a), 0.98 (3H, d, *J* = 6.4 Hz); ^{13}C NMR ($CDCl_3$) δ 167.3 s, 160.9 s, 135.7 d, 133.8 d, 128.4 d, 125.7 d, 116.3 d, 69.9 d, 67.7 d, 67.2 d, 59.6 d, 42.8 t, 39.0 t, 33.7 t, 32.8 t, 31.9 t, 30.9 t, 30.8 d, 29.6 t, 21.7 q, 21.0 q; EIMS *m/z* 423 (M^+, 43), 82 (100 rel %).

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11 17-(*R*) MTPA ester: glass, ^1H NMR ($CDCl_3$) δ 5.82 (1H, brs, NH), 5.38 (1H, dt, *J* = 6.7, 4.6 Hz, H17), 4.86 (1H, m, H15), 4.14 (1H, m, H18), 3.74 (1H, m, H13), 3.36 (1H, dd, *J* = 7.9, 11.0 Hz, H19b), 3.19 (1H, dd, *J* =7.3, 11.0 Hz, H19a), 2.08 (1H, m, H16b), 1.99 (1H, m, H16a), 1.63 (2H, m, H14ab), 0.87 (3H, d, *J* = 6.7 Hz, H22); 17-(*S*) MTPA ester: glass, ^1H NMR ($CDCl_3$) δ 5.73 (1H, brs, NH), 5.34 (1H, m, H17), 4.96 (1H, m, H15), 4.02 (1H, ddd, *J* = 5.5, 7.6, 7.6 Hz, H18), 3.84 (1H, m, H13), 3.24 (1H, dd, *J* = 8.0, 11.3 Hz, H19b), 3.04 (1H, dd, *J* = 7.0, 11.3 Hz, H19a), 2.19 (1H, ddd, *J* = 3.7, 8.5, 14.7 Hz, H16b), 2.08 (1H, ddd, *J* = 5.2, 7.6, 14.7 Hz, H16a), 1.77 (2H, m, H14ab), 0.97 (3H, d, *J*= 6.4 Hz, H22).

12 **4:** IR ($CHCl_3$) 3620, 1715, 1640, 1605, 1170, 855 cm^{-1}; ^1H NMR ($CDCl_3$) δ 6.34 (1H, ddd, *J* = 8.0, 8.0, 11.6 Hz, H3), 5.93 (1H, ddd, *J* = 0.9, 4.9, 15.8 Hz, H22), 5.90 (1H, d, *J* = 11.6 Hz, H2), 5.85 (1H, m, H7), 5.83 (1H, ddd, *J* = 1.5, 6.7, 15.8 Hz, H21), 5.71 (1H, m, H6), 5.42 (1H, brs, H26), 5.32 (1H, dd, *J* = 4.9, 6.7 Hz, H20), 4.95 (1H, brs, H29b), 4.90 (1H, brs, H29a), 4.27 (1H, m, H5), 4.19 (2H, brs. H27), 4.08 (1H, m, H15), 4.06 (1H, m, H23), 3.94 (1H, m, H19), 3.82 (1H, m, H9), 3.19 (1H, dt, *J* = 2.4, 6.0 Hz, H17), 3.02 (1H, t, *J* =2.4 Hz, H16), 2.90 (2H, m, H4), 2.37 (1H, dd, *J* = 6.4, 14.4 Hz, H14b), 2.18 (1H, dd, *J* = 6.8, 14.4 Hz, H14a), 2.08 (1H, m, H8b), 2.05 (2H, m, H12b,24b), 1.98 (1H, m, H18b), 1.95 (1H, m, H12a), 1.90 (1H, m, H24a), 1.87 (1H, m, H8a), 1.77 (1H, m, H11), 1.65 (1H, m, H18a), 1.62 (1H, m, H10b), 1.59 (3H, s, H28), 1.10 (1H, ddd, *J* = 4.9, 8.5, 13.8 Hz, H10a), 0.90 (3H, d, *J* = 6.4 Hz, H30); ^{13}C NMR ($CDCl_3$) δ 165.6 s, 144.8 d, 143.8 s, 136.0 d, 131.4 s, 128.6 d, 125.2 d, 124.8 d, 122.5 d, 119.7 d, 114.1 t, 77.0 d, 73.0 d, 72.2 d, 70.6 d, 67.4 d, 66.5 d, 65.8 t, 60.7 d, 52.4 d, 46.4 t, 41.9 t, 38.6 t, 35.7 t, 35.4 t, 35.2 t, 31.0 t, 27.0 d, 23.0 q, 20.0 q.

13 This experiment confirms the absolute configuration of isolaulimalide (**5**) as 5*R*, 9*S*, 11*S*, 15*S*, 16*S*, 17*R*, 19*S* 20*S*, 23*S*.