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LATRUNCULINS: NMR STUDY, TWO NEW TOXINS AND A SYNTHETIC APPROACH

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Abstract - A complete ^{1}H and ^{13}C NMR assignment of one of the latrunculins (B) was accomplished with the aid of 2D NMR COSY and CH shift-correlation experiments. The various H-H coupling constants have been determined and a conformation of the macrolide and the tetrahydropyran (THP) ring suggested on basis of the J-values and measured NOE's. The absolute configuration of latrunculin-A(1) was determined on the grounds of its earlier X-Ray analysis, and a chemical degradation to a known compound. Two novel latrunculins, -C(3) and -D(4), were isolated from the Red Sea sponge L. magnifica and their structures elucidated. Starting with L-cysteine a synthon for the latrunculins has been synthesized.

Latrunculin-A(1) and latrunculin-B(2) are two fish toxins which were isolated from the Red Sea sponge Latrunculia magnifica[1]. The structure of 1 was established by X-Ray analysis of a suitable crystalline derivative. The interesting biological activity of these toxins, namely, inter alia their specific activity on actin, a cytoskeletal protein[2], brought us to the study of the conformation of the molecule. Therefore, we have undertaken a complete ^{1}H and ^{13}C NMR investigation of one of the latrunculins i.e. compound 2.

Previously, while elucidating the structure of the latrunculins we have made the assignments of all functional moities protons and carbon atoms, however, the methylenes, except for CH_2-17, as well as the methine H-8 remained undetermined.

The present NMR study started with the assignments of all the protons. Intensive double irradiation experiments gave good indications for the locations of all the protons of compound 2, assignments which were confirmed by a 2D-NMR COSY experiment (see Fig.1). Additionally, COSY experiments with the emphasis on long range coup lings established many of the protons 1-3 relationships as shown in Fig.2. The correlations deduced from the various COSY experiments together with the conclusions derived from the C-H shift correlations, vide infra (Fig.3 & 4) are in full agreement with the structure of latrunculin-B suggested on basis of comparison with latrunculin-A (for which the X-Ray analysis has been performed)[1]. Actually, it can be seen that presently on the grounds of the functional groups identification (by IR, UV and mass spectrum) and the 2D-NMR data the complete structure of 2 could have been resolved independently.

After completing the proton assignments the carbon atom resonances have been determined by a C-H shift correlation (Fig.3). Additional C-H shift correlation experiments adjusted for emphasizing the long-range couplings (^{2}J and ^{3}J) confirmed

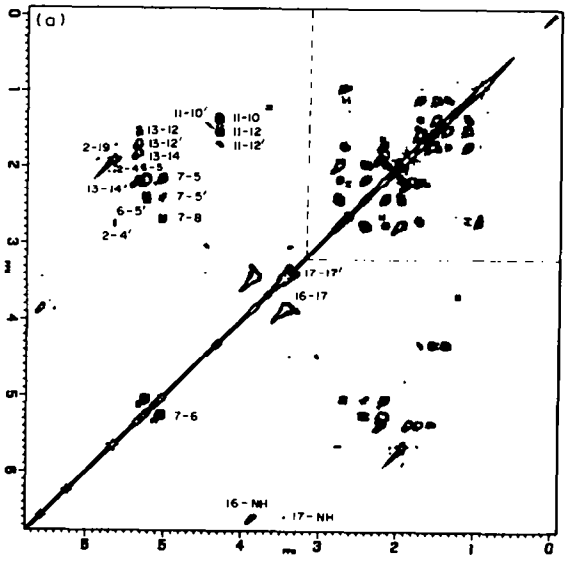


Fig. 1a: Contour plot of a 360 MHz ^1H delayed COSY-45 experiment of 0.25M latrunculin-B (2) in $CDCl_3$.

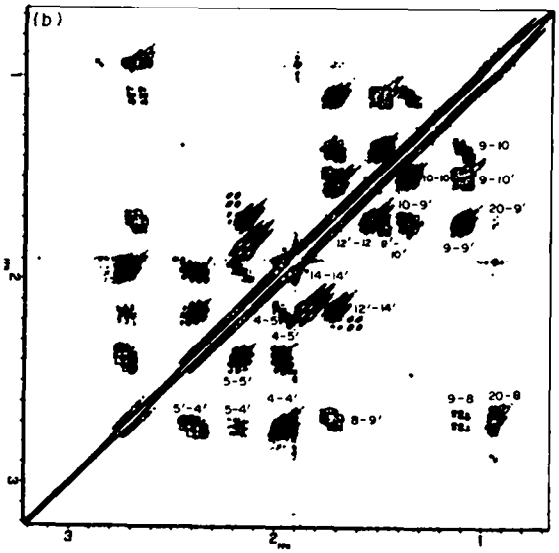


Fig. 1b: Expanded aliphatic region of the COSY spectrum illustrated in Fig.1a.

further the proposed resonance assignments of the molecule (see Fig.4). All the assignments are in full agreement with the previously proposed ones on the grounds of the chemical shifts[1b].

Next, for the conformational analysis, the H-H coupling constants had to be measured. We started with a homonuclear J-resolved spectrum, however, this spectrum did not give more than the already known couplings of the functional group protons, because of the distortions of the second-order systems of the methylenes (except for H-17, 17').

Intensive double irradiations, on the other hand, assisted by difference-spectra (DDS), difference NOE and PRFT experiments, afforded all J-values as summarised in Table 1.

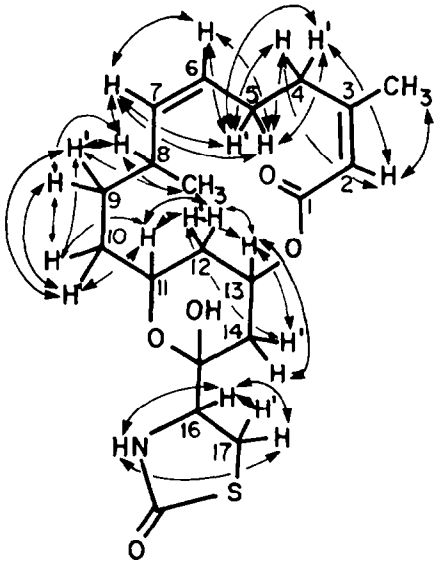


Fig. 2: Summary of the COSY experiment.

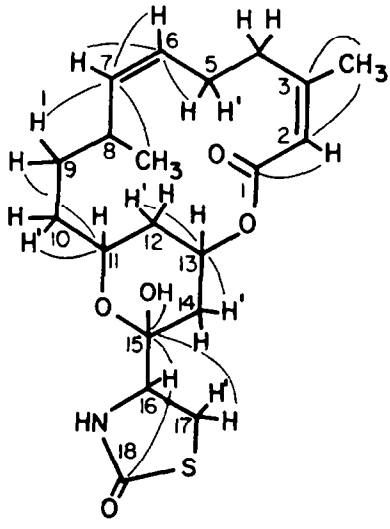


Fig. 4: Summary of the long-range C-H shift correlations.

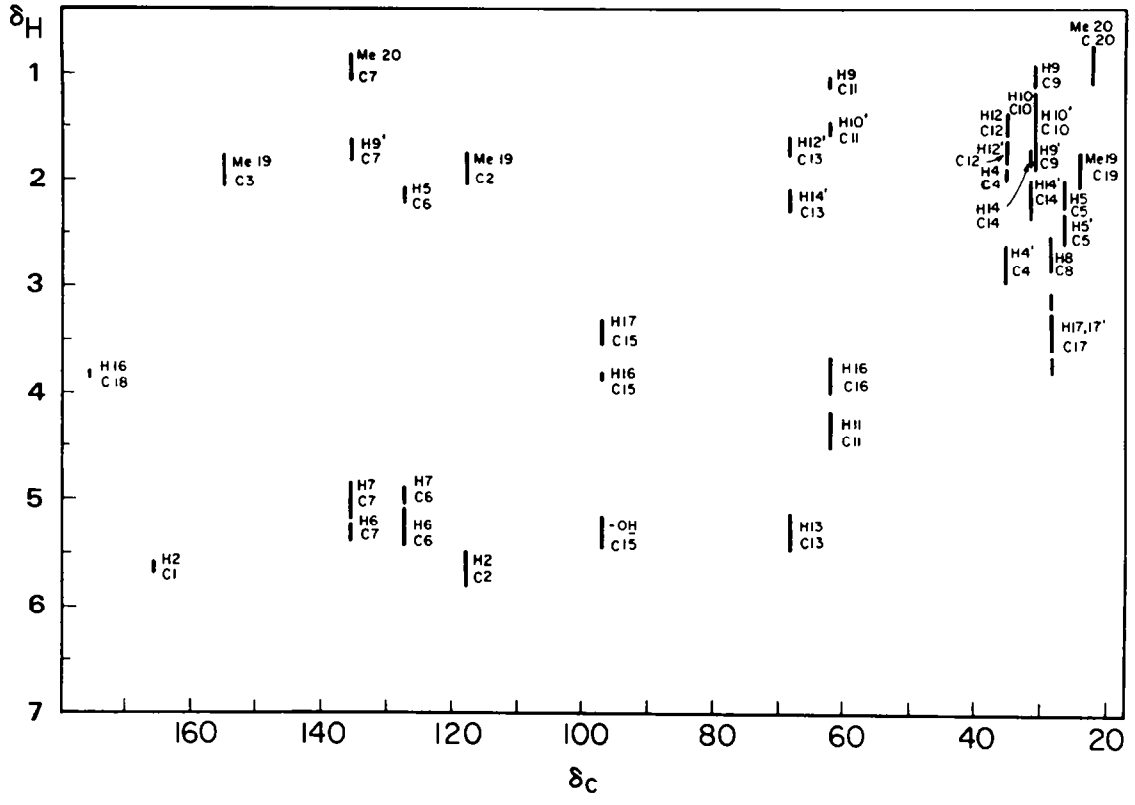


Fig. 3: Contour plot of a 360-90 MHz ^{1}H-^{13}C shift correlation experiment of 0.25M latrunculin-B (2) in $CDCl_3$.

Although the macrolide of latrunculin-B(2) is a 14-membered ring its conformational mobility is expected to be quite restricted because of the two double bonds and the THP ring which is part of the macrocycle (this is similar to other macrocycles like the cembrenes, also possessing a 14 membered ring which embodies 3 double bonds or other restricting groups like epoxides or lactones). Furthermore, transannular interactions within the medium-size ring are expected to enforce additional constraints on the macrolide and influence its conformation.

The conformation of latrunculin-B was studied by measurements of:

a. the J-values and more particularly the couplings between protons in the C_4-C_5 and C_8-C_{10} vicinities - the junctions between the restricting sites, and

b. NOE's between spatially close protons.

Coupling constants of 3-4.5 Hz between protons: 4,5; 4',5'; 9,10; 9,10'; 9',10' & 10,11 and J-values of 11-13 Hz between protons 4,5'; 4',5; 8,7; 8,9; 9',10 & 10',11 (see Table 1) point clearly to one major preferred conformation in which the dihedral angles between the former pairs of atoms are ca. 50° - 70° and the angles between the latter pairs 160° - 180°.

Observation of a Dreiding model shows that the above dihedral angles, together with the two constraining double bonds and the THP ring, substantially define the conformation of the macrolide of 2.

Further conformational information could be derived from NOE measurements. NOE's between well separated resonance lines revealed the spatial proximity of the following pairs of protons: 2,13; 2,19; 4,6;5,6; 6,7; 7,9; 7,9'; 8,11; OH, 11; OH, 16; 13 with 12, 12', 14 & 14'; NH, 16; 20, with 7,8 & 9' and 16 with 17.

Of special meaning among the latter effects (which can be observed on a Dreiding

Table 1: ^{1}H-NMR Data of latrunculin-B (360 MHz, $CDCl_3$)

H		δ	m	coupling constants				
2		5.68	brq	$J_{2,19}$=1.3	$J_{2,4}$=0.5	$J_{2,4'}$=0.5		
4	pe	1.97	tdd	$J_{4,4'}$=13	$J_{4,5'}$=13	$J_{4,5}$=4.5	$J_{4,2}$<0.5	
4'	pa	2.69	tdd	$J_{4';4}$=13	$J_{4';5'}$=13	$J_{4';5}$=4.2	$J_{4';2}$<0.5	
5	pa	2.186	tddd	$J_{5,5'}$=14	$J_{5,4'}$=13	$J_{5,7}$=2.1	$J_{5,4}$=4.5	$J_{5,6}$=3.0
5'	pe	2.36	ddddd	$J_{5';5}$=14	$J_{5';4}$=12.5	$J_{5';6}$=11	$J_{5';4'}$=4	$J_{5';7}$=0.6
6		5.25	tdd	$J_{6,7}$=11.2	$J_{6,5'}$=11	$J_{6,5}$=3		
7		5.05	tdd	$J_{7,8}$=11.2	$J_{7,6}$=11.2	$J_{7,5}$=2.1	$J_{7,5'}$=0.6	
8		2.66	m	$J_{8,20}$=6.6	$J_{8,7}$=11	$J_{8,9}$=11	$J_{8,9'}$=5	
9	pe	1.106	dddd	$J_{9,9'}$=14	$J_{9,8}$=11	$J_{9,10'}$=4	$J_{9,10}$=4.5	
9'	pa	1.71	m	$J_{9';10}$=12.7	$J_{9';9}$=14	$J_{9';10'}$= 4	$J_{9';8}$=5	
10	pe	1.366	dddd	$J_{10,10'}$=14	$J_{10,9'}$=13	$J_{10,9}$=4.5	$J_{10,11}$=3	
10'	pa	1.499	dddd	$J_{10';10}$=14	$J_{10';11}$=12.7	$J_{10';9}$=4	$J_{10';9'}$=4	
11	ax	4.26	dddd	$J_{11,10}$=3	$J_{11,10'}$=12.7	$J_{11,12}$=11	$J_{11,12'}$=1.7	
12	ax	1.53	ddd	$J_{12,12'}$=14	$J_{12,11}$=11.7	$J_{12,13}$=2.3		
12'	eq	1.74	dddd	$J_{12';12}$=14	$J_{12';11}$=1.7	$J_{12';13}$=3	$J_{12';14'}$=2	
13	eq	5.34	dddd	$J_{13,12}$=2,3	$J_{13,12'}$=3.0	$J_{13,14}$=2.9	$J_{13.14'}$=3.5	
14	ax	1.89	dd	$J_{14,14'}$=14.5	$J_{14,13}$=2.9			
14'	eq	2.205	ddd	$J_{14';14}$=14.5	$J_{14';13}$=3.5	$J_{14';12'}$=2		
16		3.84	ddd	$J_{16,17}$=6.4	$J_{16,17'}$=8.6	$J_{16,NH}$=1.1		
17		3.41	dd	$J_{17,17'}$=11.4	$J_{17,16}$=6.4			
17'		3.46	dd	$J_{17';17}$=11.4	$J_{17';16}$=8.6			
19		1.90	d	$J_{19,2}$=1.3				
20		0.95	d	$J_{20,8}$=6.6				
NH		6.02	brs					

pe=pseudo equatorial, pa=pseudo axial, the identification of H-4,4' and H-5,5' is based on the viginal couplings of H-5,5' with H-6 and the allylic couplings with H-7: $J_{5',6}$=3, $\phi_{5',6}$=60°; $J_{5,6}$=11, $\phi_{5,6}$=170°; $J_{5,7}$=2.1, $\phi_{5,7}$=90° and $J_{5',7}$=0.6, $\phi_{5',7}$=0°. The identification of the C_8-C_{10} protons is based on the axial H-11 atom (see text for the J/φ relationships). Protons 4,5,9' & 10 point to the same direction as H-11ax(β) and protons 4',5',9 & 10' to the direction of H-13eq(α).

model) are the ones between:

a. H-12 & 13 - pointing to the orientation of the lactone, namely, that the CO group is in approximately the same direction as the C-O bond (β),

b. H-4 & 6 - suggesting H-4 to be parallel to H-6,

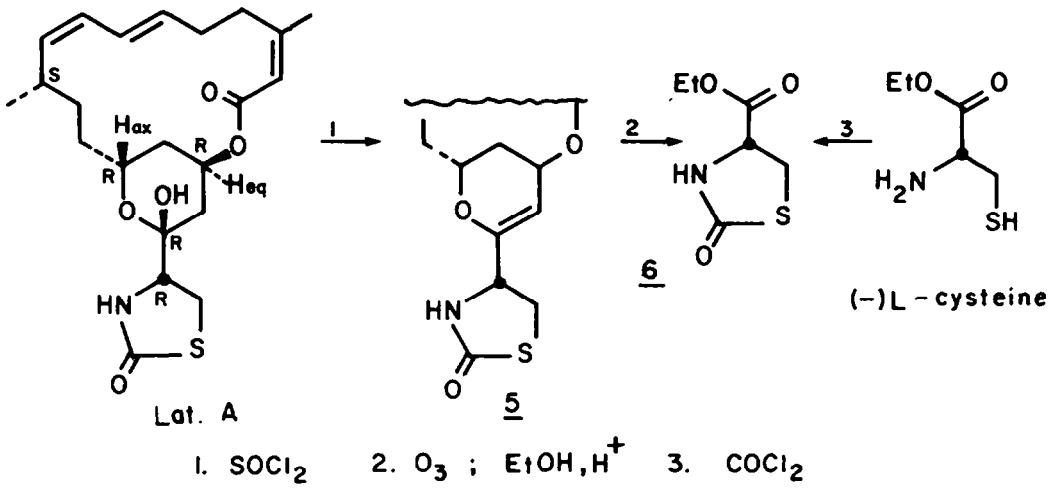
c. H-7 & 9 and 7 & 9' - proposing H-7 to bisect the H_9-C_9-$H_{9'}$ angle,

d. H-8 & 11 - pointing to a transannular proximity between H-5' and H-12' (overlapping with other protons prevented a direct NOE measurement between the latter two atoms), and

e. H-16 & OH - referring to a preferred C_{15}-C_{16} rotamer.

The coupling constants of H-11 and H-13 with their neighbours (Table 1) point clearly to their axial and equatorial conformation respectively, which together with the NOE between the OH and H-11 determine the conformation of the THP ring.

The proposed macrolide and THP conformations, of 2, resembles the conformation of 1 in the solid state [1b]. Furthermore, according to the coupling constants of the C_7-C_{11} segment, the configuration of the 20-Me group in 2 has to be the same as the 22-Me configuration in 1 (i.e. of the 8S-configuration), see below.

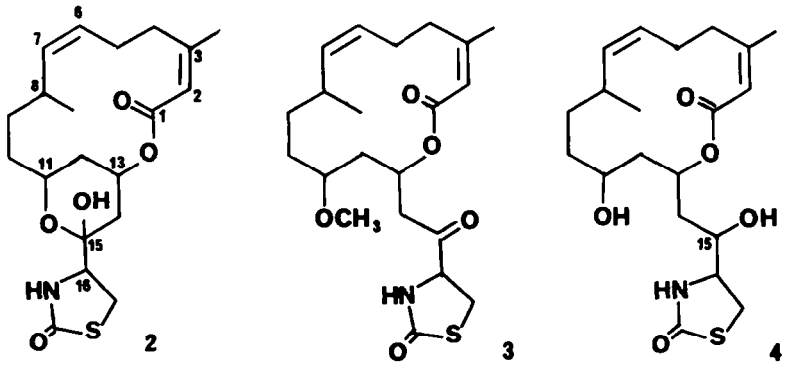


Scheme 1: Degradation of latrunculin-A (1).

In order to complete the structure of one of the latrunculins we have determined the absolute configuration of 1 by comparison of its degradation product 6 with ethyl 2-oxo-4-thiazolidinecarboxylate prepared from L-cysteine (see Scheme 1). Compound 1 was treated with $SOCl_2$/Pyridine to afford the corresponding 16(17) olefin 5[1]. Ozonolysis of the latter followed by transesterification with acidic ethanol resulted in the ethyl ester 6, which was identical in all respects, including the same negative α_D, with compound 6 prepared from L-cysteine. Based on the latter experiment and the X-Ray analysis[1] compound 1 is of the 10S,13R,15R,17R,18R absolute configuration.

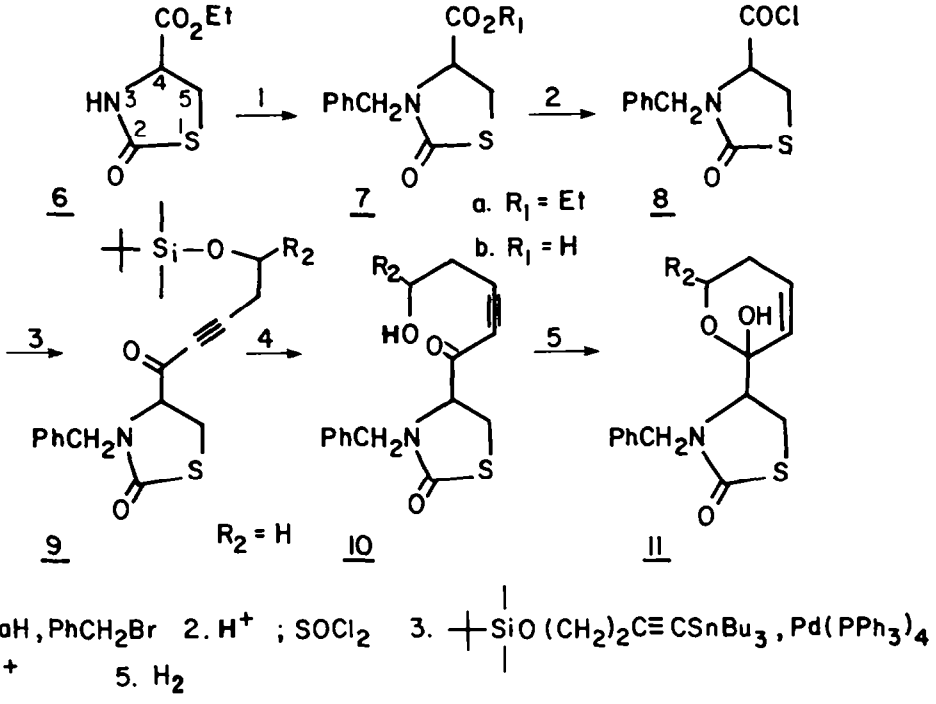
During the isolation process of latrunculin-B, in the purification stage from accompanying glycerides, we have isolated two new fish toxins designated latrunculin-C(3) and -D(4).

Latrunculin-C, a foaming oil, m/z 397(M^+) & 277(M^+-102), is slightly more polar than compound 2. The mass spectrum of 3 (the 277 peak standing for loss of the thiazolidinone moiety), the IR(3550,OH and 1680 br,2-thiazolidinone and $\alpha\beta$-unsaturated ester) and especially both the ^1H and the ^{13}C NMR spectra (see Experimental) suggest for 3 a closely related structure to latrunculin-B(2). Most significant in the ^{13}C NMR spectrum of 3 was the absence of the hemiketal carbon of 2 (a singlet at δ 97.7), and the appearance of a new methinoxy doublet at δ 69.6, both pointing



Scheme 2: Latrunculins-B(2), -C(3) and -D(4).

clearly to the reduction of the 15-keto group (which in 2 appears as the hemiketal) to the corresponding alcohol (Scheme 2). The absence of the THP ring causes, as expected, changes in the ^{13}C-NMR signals of the C_{11}-C_{16} segment (see Experimental). Apart from the latter differences all the rest of the C-atom resonances remain essentially unchanged (±0.5ppm). The proton NMR, of the common functional sites, is in full agreement with that of latrunculin-B. Changes can be seen in the H-11 & H-13



Scheme 3: A synthetic approach towards latrunculin-B

multiplets due to opening of the THP ring and also as expected, in the 4 ppm region where the additional methinoxy (H-15) appears. As the latter proton was suspected to overlap with H-16 at δ 3.85, we have prepared the 11,15-diformate of 3 (compound 3b) and indeed, in the NMR spectrum of this compound H-16 is separated from H-15 (which is down field shifted by ca. 1.4 ppm - see Experimental). The H-16 signal, of 3b, appears now as a broadened quartet, coupled with H-17,17' and H-15 as shown by double irradiations, thereby confirming the reduction of the 15-carbonyl group in 3. Finally, unequivocal proof for the structure of compound 3 was obtained by its synthesis from latrunculin-B(2). Reduction of 2 with $NaBH_4$, afforded the two possible epimeric 15-alcohols, one of which was identical in all respects with latrunculin-C(3).

The second new compound, latrunculin-D(4) is of almost the same polarity as 2 and additional accompanying glycerides; it was therefore very difficult to purify the compound. Characteristic in the ^{1}H-NMR spectrum of 4, was a methoxy group (δ 3.68s) and changes in the H-11 & H-13 multiplets which point, as with 3, to the opening of the THP ring. However, in contrast to compound 3, the H-16 resonance appeared as a triplet (rather than a broadened quartet in 3) coupled with H-17 & 17' only. On grounds of biosynthetic considerations and the NMR data we suggest latrunculin-D to be the 11-methoxy derivative of 2 (Scheme 2).

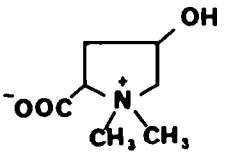
A synthetic approach towards the synthesis of a synton of the latrunculins is shown in Scheme 3. The preparation of the bicyclic 2-thiazolidinone-tetrahydropyran

system (11) is of particular importance as, in slight modifications, it is a starting material for the synthesis of 2 ($R_2 \neq H$) as has earlier been shown in a suggested retrosynthesis[1b], and secondly, as a model for studying the chemical behaviour of this functional moiety of the latrunculins. The synthesis starts with L-cysteine which is believed by us to be also the biogenetic precursor. Reacting the ethyl ester of cysteine with phosgene afforded the known thiazolidinone 6[3]. Protection of the NH group by benzylation (7a), and treatment of the corresponding acid 7b with $SOCl_2$ resulted in the acyl chloride 8. The latter was coupled[4] with the silyloxy stannane, $Bu_3SnC \equiv CCH_2CH_2OTBDMS$, in the presence of $Pd(PPh_3)_4$ to give compound 9. Compound 9 is a good starting material for the preparation of 11 or other 4-substituted 6-hydroxy-6 [4'-(2'-oxothiazolidinyl)]tetrahydropyran derivatives.

Treatment of 9 with acidic acetone cleaved the silyloxy group to give the corresponding alcohol 10. Compound 10 upon partial hydrogenation over Lindlar's catalyst, afforded the desired compound 11. The latter (11) exists in an almost 1:1 mixture of the two possible diastereomeric hemiketals - as could be judged from the NMR spectrum (see Experimental), together with ca. 5% of the open hydroxy ketone. Compound 11 was expected to be in equilibrium with the $\alpha\beta$-unsaturated ketone and to undergo Michael additions. These reactions together with the conformational analysis of 11 will be the subject of a forthcoming report.

Lastly, we want also to report the isolation of two isomeric betaines, betonicin and turicin (the cis and trans N,N-dimethyl-4-hydroxy proline betains)[5] from the methanol extract of L. magnifica.

The two compounds can be easily identified by their ^{1}H-NMR spectrum (δ 4.37 dd(J=7.5, 10.4), 4.06dd (J=6.3,12.9), 3.53dd(J=3.8, 2.9), 3.40s(3H), 3.30m, 3.12s(3H), 2.69 ddd and 2.40 m for one isomer and small changes for the other).



Since the identification of the latter betains from L. magnifica we have identified them in several other sponge methanol extracts.

EXPERIMENTAL SECTION

Infrared spectra were recorded on a Perkin-Elmer Model 177 spectrophotometer. Optical rotations were measured on a Perkin-Elmer Model 141 polarimeter using a 10-cm microcel. NMR spectra were recorded on a Bruker AM-360 NMR spectrometer equipped with an ASPECT 3000 computer. Bruker ASPECT 3000 2D-NMR programs have been used for recording the 2D spectra; all chemical shifts are reported with respect to TMS (δ=o). Low resolution mass spectra were recorded on a Dupont 21-491B mass spectrometer. High resolution mass spectra were recorded on a Varian MAT 731 mass spectrometer. Melting points were determined on a Thomas Hoover Capillary m.p. apparatus and are reported uncorrected. All solvents used were either spectral grade or freshly distilled.

The 2D-NMR experiments were measured on a 0.25 M sample of latrunculin-B(2) in $CDCl_3$, at 298°K. The H-H shift correlation experiment was performed with a delayed COSY 45 sequence. The presented two-dimensional map is composed of 512 x 2K data point spectra. A 1 sec. recycle delay was allowed between each pulse sequence, and an extra delay of 0.6 sec was inserted before the evolution and the detection periods. Quadrature detection was applied in both dimensions using the 16 step phase cycling for N-type peak selection. Data were multiplied with sine bell shaping function, zero filled to 1K x 2K and then Fourier transformed and symmetrized.

The ^{1}H-^{13}C shift correlation experiment was performed with the regular heteronuclear shift correlation pulse sequence. The two-dimensional map is composed of 300 x

2K data point spectra. A 1 sec recycle delay was allowed between each pulse sequence. The time for the development of the polarization transfer, $\tau_1=2J^{-1}$, and the refocusing time for antiphase multiplet components, $\tau_2=4J^{-1}$, were adjusted to give maximum enhancement for J_{CH}=144 Hz. Quadrature detection was applied in both directions using 8 step phase cycling for N-type peak selection. Data were multiplied with sine bell shaping function, zero filled to 1K x 2K and then Fourier transformed.

Degradation of latrunculin-A (1) to give ethyl 2-oxo-thiazolidine-4-carboxylate

Latrunculin-A (100 mg) was treated with $SOCl_2$ in pyridine as described earlier[1] to give the 16(17) olefinic derivative (5, 75 mg). Ozonolysis of 5 (75 mg) in CH_2Cl_2 (8 ml) at -70° for 2 minutes was followed by reductive work-up, namely, treatment with Zn (100 mg) in a mixture of THF: 10% HOAc (2: 1, 8 ml). After 16 hrs CH_2Cl_2 was added (50 ml) the solution washed to neutral, dried and evaporated. The residue was taken into EtOH (8 ml) and a few drops of HCl in EtOH added. After 48 hrs the solution was neutralized, the ethanol evaporated and the residue filtered through a short Sephadex LH-20 column (eluted with $CHCl_3$:MeOH 1:1). Further purification of the product was achieved by chromatography on a silica gel column (eluted with petrol-ether ethyl-acetate 1:1) to afford ethyl 2-oxo-thiazolidine-4-carboxylate, $[\alpha]_D^{20}$ -35° identical with a sample prepared from L-cysteine according to Maclaren[3]; an oil, λ_{max}272(ε=500), $[\alpha]_D^{20}$-42° (c 2.0,$CHCl_3$), IR(neat) 2970,1730,1670br,1510 cm^{-1}; δ_H7.19bs(NH),4.50dd (J=8.2,4.6),4.26q(J=7,2H),3.75dd(J=11.5,8.2),3.58dd(J=11.5,4.6),1.31t(J=7,3H).

Collection, Extraction and Isolation of Latrunculin - C(3) and - D(4)

The sponge specimens were collected at a depth of 20-30 m in the northern part of the Gulf of Eilat. After freeze-drying, the material (100 gr) was extracted with petroleum ether for 24 hrs. The crude extract, after evaporation, was chromatographed in batches of 2-4 gr on a LH-20 column (2:1:1 petroleum ether-$CHCl_3$-MeOH). The Latrunculins-containing fractions were combined and rechromatographed on LH-20 and silica gel-H columns. Elution from the latter column with 40-50% ethyl acetate in petroleum ether afforded pure 2 and 4 (ca. 90% pure) and with 50-70% ethyl acetate latrunculin-C (3).

Latrunculin-C (3); a foaming oil, λ_{max}(CH_3OH) 212(ε=17000); IR($CHCl_3$)3520,2910, 1675br,1205cm^{-1}; m/z 397(2),379(4),295(13),277(70); δ_H($CDCl_3$): 5.62bs(H-2),5.23dt (J=11.2,3.5;H-6),5.09m(H-13),5.02t(J=11,H-7),4.08m(H-11),3.84m(2H,H-15&16),4.48dd(J= 11,7.5,H-17),4.43dd(J=11,5.2,H-17'),1.90bs(Me-19),0.97d(J=6.4,Me-20); δ_C($CDCl_3$): 175.7s,167.2s,155.3s,135.9d,127.7d,118.2d,70.7d,69.6d,65.6d,59.1d,40.7t,35.6t,34.9t, 34.8t,31.8t,30.9t,29.1t,25.9t,24.2q,21.9q.

Latrunculin-D (4), an oil, λ_{max}(CH_3OH) 212(ε=17000); IR($CHCl_3$) 2910,1700,1675br, 1205 cm^{-1}; m/z 393(1),291(2); δ_H($CDCl_3$): 5.67bs(H-2),5.34dt(J=10.5,7.7,H-6),5.12t(J= 10.3,H-7),5.10m(H-13),3.81m(H-11),3.76t(J=7.6,H-16),3.68s(OCH_3),3.43m(H-17,17'),1.90d (J=1.3,Me-19),0.93d(J=6.7,Me-20).

Reduction of Latrunculin-B to Latrunculin-C(3)

Latrunculin-B (2, 30 mg) in MeOH (5 ml) was reduced with $NaBH_4$ (5 mg) at r.t. for 2 hrs. The usual work-up afforded a mixture of the two epimeric 15-ols. One of the compounds possesses exactly the same NMR spectrum as compound 3. The epimeric 15-ol, which was only partly separated from 3 exhibited the following resonance lines: 5.67s(H-2),H-6,7,11 - identical with those of 3, 5.21m(H-13),3.76m(2H,H-15&16),3.32dd (J=11,7.7,H-17),3.18dd(J=11,8.2,H-17').

Latrunculin-C diformate (3b)

Latrunculin-C (10mg) was left for 72 hrs in a solution of formic acid (5ml). Evapo-

ration of the acid afforded the diformate as an oil; IR($CHCl_3$) 2910,1720,1625br, 1205 cm^{-1}; δ_H($CDCl_3$): 8.14s(OCHO),8.10s(OCHO),6.00(NH),5.67s(H-2),5.25m(H-15),5.00m (H-11),5.30dt(H-6),5.04t(H-7),4.08q(H-16),3.52dd and 3.23dd(H-17,17'),1.96s(Me-19), 0.95d(J=2,Me-20); m/z 453(M^+) and 407(M^+-46).

Ethyl-N-benzyl-2-oxo-thiazolidine-4-carboxylate (7a)

To a 150 ml double-necked round bottomed flask, equipped with a magnetic stirrer, was added 0.305gr (7.0 mmol) of 55% dispersion of sodium hydride in mineral oil. The sodium hydride was washed three times by decantation with dry benzene and then covered with 50 ml of dry benzene. A solution of 6 (1.11 gr, 6.36 mmol) in 25 ml of dry benzene was added dropwise at room temp., while stirring the NaH solution, over 10 min, and the H_2 which evolved measured in a glass column. After 1 equivalent of H_2 was evolved (10 additional min), 1.25 ml (10.5 mmol) of benzyl bromide was added by injection, the reaction mixture refluxed for 1.5 hrs, cooled to room temp. and then poured into 50 ml water. The benzene layer was separated, dried over $MgSO_4$ and distilled in vacuo. The crude material was purified by chromatography over a silica gel-H column using 10% EtOAc in petroleum ether to give 7, (792 mg, 47%); an oil; $[\alpha]_D^{25}$-58° (c 0.05,$CHCl_3$), IR(neat) 3100-2880,1736br,1675br,1445,1190br,1025,695 cm^{-1}; δ_H($CDCl_3$): 1.29t(J=7,3H),3.35dd(J=11.4,3.0,1H),3.50dd(J=11.4,8.5,1H),4.05d(J=15,1H), 4.14dd(J=8.5,3.0,1H),4.23q(J=7,2H),5.14d(J=15,1H),7.22-7.36m(5H); δ_C($CDCl_3$): 14.04q, 28.91t,47.85t,59.42d,62.02t,127.95d,128.27d,128.79d,135.63s,168.52s,169.70s; m/z 266 (M^++1,32%),237(M^++1-Et,13),192(M-CO_2Et,100) and 91(65).

N-Benzyl-2-oxo-thiazolidine-4-carboxylic acid (7b).

A solution of 7a (1.2gr,4.5mmol) in a mixture of HOAc (4ml) and HCl conc. (2ml) was heated to 100°C for 30 min. The acetic and hydrochloric acids were then removed by evaporation and the oily residue taken into CH_2Cl_2 (20ml). The acid (7b) was purified by extraction into 5% NaOH (2X20 ml), acidification to pH 1 and reextraction into CH_2Cl_2 (2x20ml). This was a white solid, m.p. 95°-97°C; IR(KBr)3500,1680br cm^{-1}, δ_H($CDCl_3$): 7.40m&7.26m(Ph),6.11br(CO_2H),5.23d(J=15) and 4.07d (J=15)-PhCH_2,4.21dd(J= 8.5,2),3.57dd(J=11.5,8.5),3.43dd(J=11.5,2); δ_C: 59.0d,48.1t,29.0t; m/z 237(M^+,23%), 192(M^+-CO_2H,100),91(21).

Compound 8 was prepared, in situ, by heating a solution of 7b (595 mg) in $SOCl_2$ (4 ml) to 50°C for 1 hr. The excess of the $SOCl_2$ was removed under vacuo and the residue taken into 1,2-dichloroethane.

4-(tert-Butyldimethylsilyl)-oxy-1-butyne

To a solution of 5.25 gr (75 mmol) 3-butyn-1-ol and 11.25 gr (75 mmol) tert-butyldimethylsilyl chloride in 60 ml methylene chloride (dried over calcium hydride) at 0°C, 9gr (90 mmol) triethylamine was added dropwise keeping the temperature at 2°-5°C. Then 100 mg (1.5 mmol) imidazole was added, the ice bath removed, and the mixture stirred for an additional 1 h at room temperature. The mixture was then diluted with 60 ml methylene chloride and washed with 60 ml 1N HCl. The organic layer was dried over $MgSO_4$ and the solvent removed under vacuo. Distillation of the residue gave 10.5gr(76%) of colourless liquid b.p. 78°-81°C/20mm; m/z 184(1%); IR(neat) 3310, 2110 cm^{-1}; δ_H(CCl_4,60MHz): 0.00s(6H),0.77s(9H),1.70t(J=2, ≡CH),2.20dt(J=7,2,2H),3.50t (J=7,2H).

[4-(tert-Butyldimethysilyl)-oxy-1-butynyl]-tributyl stannane.

A solution of 4-(tert-butyldimethylsilyl)-oxy-1-butynyl lithium in THF was prepared by dropwise addition of 13.5 ml (20 mmol) butyl lithium, in hexane, 1.5 M, to 3.7 gr (20 mmol) 4-(tert-butyldimethylsilyl)-oxy-1-butyne in 15 ml THF, at 0°C,

during 25 min. Chlorotributyl stannane 6.5 gr (20 mmol) in THF (5 ml) was then added dropwise to the organo lithium solution. The mixture was stirred overnight at r.t., then diluted with CH_2Cl_2(40 ml), washed with water, dried over $MgSO_4$ and the solvents removed under vacuo. Distillation afforded 4.8 gr (50%) of the stannane as a colourless liquid; b.p. 135°-140°C/0.1mm, IR(neat)2130 cm^{-1}; $\delta_H(CDCl_3)$: 3.65t(J=7, 2H),2.35t(J=7,2H),1.80-0.80m(30H),0.89s(9H),0.05s(6H); m/z 415.8(M^+-Bu,3.7),357.1 (M^+-2Bu,58),301.3(M-3Bu,30).

5-(tert-Butyldimethylsilyl)-oxy-1-(4'-N-benzyl-2'-oxo-thiazolidinyl)-2-pentyn-1-one (9).

To a solution of compound 8 (600 mg) in 10 ml CH_2ClCH_2Cl (dried over P_2O_5) was added 1.4 gr (3 mmol) of the above described butynetributyl stannane and 53 mg (0.045 mmol) tetrakis(triphenylphosphine) palladium(0)[4]. The mixture was refluxed for 1 hr, then cooled to r.t., ether (40 ml) added and vigorously stirred for 5 min with a saturated solution of KF (50 ml). The precipitate of Bu_3SnF was removed by filtration and the organic layer dried over $MgSO_4$. The residue after evaporation of the solvents was chromatographed on a short silica gel-H column, eluted with ethyl acetate, petroleum ether 1:9 to afford 385mg (38%) of 9; an oil $[\alpha]_D^{25}$ -65° (c 0.07, $CHCl_3$); m/z 403(M^+,0.3),388(M^+-Me,0.4),346(M^+-Bu,26),192($PhCH_2NCOSCH_2CH^+$,100),91(55), IR(neat) 2210,1720 cm^{-1}; $\delta_H(CDCl_3)$: 7.3m(5H),5.21d(J=15,PhCH),4.14dd(J=9,3, the ABX of the thiazolidinone), 3.96d(J=15,PhCH'),3.78t(J=7,CH_2O),3.52dd(J=11.7,9,ABX),3.44dd (J=11.7,3.1,ABX),2.62t(J=6,$\equiv CCH_2$); δ_C 183.5s,171.4s,135.4s,128.8d,128.2d,127.9d, 97.2s & 79.1s(C≡C),66.1d,47.7dd(the thiazolidinone),60.3t(CH_2O),28.1t(CH_2N),25.6q, 23.5t,18.0s,-5.5q.

6(4'-N-benzyl-2'-oxo-thiazolidinyl)-6-hydroxy- Δ^4-tetrahydropyran(11)

The TBDMS derivative of 9 (136 mg, 33 mmol) was heated for 1 h in a mixture of acetone (4ml) and 0.2M HCl (4ml) at 50°-60°C. The acetone was then removed under vacuo and the residue taken into CH_2Cl_2 (10ml). The organic phase was washed to neutral, dried over $MgSO_4$ and evaporated to give the alcohol 10 (90mg); an oil,$[\alpha]_D^{25}$ -50°(c 0.02,$CHCl_3$); IR($CHCl_3$) 3500,2200,1670br,1160 cm^{-1}, $\delta_H(CDCl_3)$: 7.38&7.28m(Ph), 5.25d(J=15,PhCH),4.15dd,(J=8,3),4.04d(J=15,PhCH'),3.84t(J=6.5,CH_2O),3.58dd(J=11,8), 3.37dd(J=11,3),2.70t(J=6.5,$CH_2C\equiv$); m/z 290(M^++H,0.7),192(100),91(37). Hydrogenation of compound 10 (30mg) in acetone (5ml) over Lindlar's catalyst (8mg), afforded the olefin 11 (25mg) as an oil; $[\alpha]_D^{25}$-55°(c 0.04,$CHCl_3$); IR($CHCl_3$) 3500,1670,1600 cm^{-1}, $\delta_H(CDCl_3$ mixture of two hemiketals): 7.4-7.2m(Ph),6.204dd(J=10.2,1)&6.185bdd(J=10.2,1; H-5),6.009ddd(J=10.2,2.7,1.2)&5.876ddd(J=10.2,2.7,1.2)H-4,5.190d(J=14.8),5.060d(J= 14.7),4.535d(J=14.8)&4.408d(J=14.7)$PhCH_2$,3.93m and 3.85-3.75m(H-2,2')3.93&3.10-3.40m (the thiazolidinone),2.31&1.93m(H-3,3'); m/z 292(M^++1,2.5),273(M^+-H_2O,2.6),192(100), 99($C_5H_7O_2$,6),91(51).

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References

1. a. Y. Kashman, A. Groweiss, and U. Shmueli, Tetrahedron Lett. 21, 3629 (1980).
   b. A. Groweiss, U. Shmueli and Y. Kashman, J. Org. Chem. 48, 3512 (1983).
2. I. Spector, N.R. Shochet, Y. Kashman, and A. Groweiss, Science 4584, 493 (1983).
3. J.A.Maclaren, Aust. J. of Chem. 21, 1891 (1968).
4. W.L. Marshall and K. Teng, J. Org. Chem. 47, 2549 (1982).
5. The Merck Index, Ninth Edition, 1976; 1218 and 9473.