Tetrahedron Letters,Vol.27,No.25,pp 2797-2800,1986 0040-4039/86 $3.00 + .00
Printed in Great Britain Pergamon Journals Ltd.

# JASPLAKINOLIDE, A CYCLODEPSIPEPTIDE FROM THE MARINE SPONGE, JASPIS SP.

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**SUMMARY:** A new cyclodepsipeptide comprised of three amino acids, and an oxy-trimethyl-nonanoyl group, has been characterized which has antifungal and anthelminthic bioactivity.

We have encountered several soft-bodied sponges, during past Tonga - Fiji expeditions,[1] whose crude extracts are active against yeast. As one example, the crude extract of a small 1984 collection of a Jaspis sp. which was bright orange (order, Astrophorida; family, Jaspidae),[2] from the Island of Benga, Fiji, showed a 33 mm growth inhibition zone, at 100 μg/mL, against Candida albicans. Recollection of this sponge yielded Jasplakinolide (1) which showed selective antimicrobial activity. A minimum inhibition concentration (mic) and minimum lethal concentration (mlc) of 25 μg/mL each was observed against C. albicans, and the in vivo topical activity of a 2% solution of 1 against a Candida vaginal infection in mice was comparable to that of miconazole nitrate, whereas no activity was observed, at 100 μg/ 13 mm disk, against E. coli, Pseudomonas aeruginosa, S. aureus, Streptococcus pyrogenes, Mycoplasma sp., or T. mentagrophytes. Jasplakinolide exhibited an in vitro $ED_{50}$ < 1 μg/ml against the nematode Nippostrongylus braziliensis, and complete in vitro cell toxicity, against larynx epithelial carcinoma at 0.32 μg/ml, and human embryonic lung at 0.01 μg/ml.[3] The chemical structure of Jasplakinolide (1) will now be reported.

Jasplakinolide was obtained by methanol extraction of the freshly collected sponge (3 Kg wet weight), and, by $^{13}C$ NMR, it was a major component of the crude viscous oil. Solvent partitioning of the crude extract between methanol (wet) and a series of: hexanes, $CCl_4$, and $CH_2Cl_2$ left reasonably pure 1 (1.4g, from 8.5g of crude oil) in the $CH_2Cl_2$ fraction. It was then easily purified (0.9g, 63% yield) by reverse phase HPLC (10μ ODS column, acetonitrile).

Structure elucidation of 1 ($[\alpha]_D$ = +35° (c 3.62, $CH_3OH$), UV $\lambda_{max}$ ($CH_3OH$) = 281 (5400), 290 (4100), IR ($CDCl_3$) 3400-3100, 1710, 1660, 1630) was begun by intensive study of spectrometric data. The molecular formula, $C_{36}H_{45}N_4O_6Br$, was established by $M^+$+1 and $M^+$+29 doublets in the high resolution chemical ionization (methane) mass spectrum and by the C and H count obtained from NMR. The $^{13}C$ NMR ($CDCl_3$) revealed its functional groups, as follows: (a) two kinds of carbonyls including one ester (δ170.8s, 70.7d) and three amides (δ175.3s, 174.7s, 169.1s, 55.7d, 49.2d, 46.1d); (b) a 2,3-disubstituted indole (δ110.4s, 109.2s); (c) a p-substituted phenol (δ155.9s, 131.7s); and (d) an E -C(H)=C(Me)- array (δ128.3d, 133.8s, 18.5q). Comparison of the molecular unsaturation number to the count of unsaturation sites in the above functionalities indicated that one additional ring was present which could contain the ester and the three amides. Additional NMR results (Table 1) and especially two-dimensional homo and hetero COSY NMR data (Table 2) along with mass spectral CI fragment peaks (Scheme 1) revealed the four sub-structures containing the above functional groups. A tetraproprionate group, 8-oxy-2,4,6-trimethyl-4E-ene-nonanoyl (OTMN), was identified by interpreting direct and long range coupling correlations.

Tracking the COSY results beginning at H-15 (see numbering in **1**) revealed that the carbon chain extended from it, in one direction to the δ1.05 Me-33, and in the other to the δ175.3 C=O (C-8). The cleavage of $C_{12}H_{20}O$ in the high resolution CI mass spectrum provided added confirmation of the OTMN array. An alanine (ALA) group was identified by NMR COSY peaks which showed that NH-b was adjacent to a CH-$CH_3$ and the latter methyl correlated to the δ174.7 C=O (C-6). Two $^1H$ NMR ABX patterns, which indicated substructures **A** or **B**, were associated with the remaining two amino acids. A 2-bromo-3-alkylindole was noted by similar peaks in the vinyl $^{13}C$ NMR region between **1** and models **3**[4a] and **4**[4b]. Substructure **A** was firmly established for the 2-bromo-N-methyltryptophan (BrMeTRP) by the $^1H$-$^{13}C$ (J=9) COSY peaks from H-20 to C-5, C-21 and C-28, and mass spectral fragmentation loss of $C_{12}H_{13}N_3OBr$ (m/z at 414.2250) and a $C_{12}H_{14}N_3OBr$ (m/z at 296/298) fragment. The $^{13}C$ NMR peaks associated with the remaining amino acid were analogous to those of tyrosine methyl ester (**2**), and a $C_9$ mass spectral fragment was observed at m/z = 147. Initially, we assigned substructure **A** to the tyrosine based upon biogenetic considerations and results of the acid hydrolysis of **1** (see below).[5] However, definitive evidence for **B** was obtained by the LAH reduction product **5** (MS: FAB m/z = 713, 715, $C_{36}H_{49}N_4O_6Br$ + H) which showed $^1H$ NMR triplet coupling at H-1 from H-2 and identical coupling at H-3 in both **1** and **5**. The hydrolysis of **1** with 6 N HCl gave a complex mixture which was analyzed by HPLC. This included a cluster of peaks with identical retention time versus that of α-tyrosine, and a single peak whose retention time was close to that of tryptophan. An additional hydrolysis product, 8-hydroxy-2,4,6-trimethyl-4E-ene-nonanoic acid was purified by HPLC and identified by $^1H$ NMR. The 1 tyrosine OH was esterified with acetic anhydride in pyridine yielding **6** and the OTMN C=C was transformed by $OsO_4$ into a mixture (85:15) of two cis diol diastereomers **7**.

The amino acid connectivity as -ALA-BrMeTRP-βTYR- was set by observing several direct or long range NMR COSY peaks, and by mass spectral fragments. Support for the C=O assignments shown in Table 1 came from unambiguous links that were established from protons in each sub-structure to each carbonyl group carbon. The most definitive results (Table 2) included: both Me-30 protons and H-10' correlated to C-8; both H-2,-2' correlated to C-1; both Me-29 and Me-34 protons correlated to C-6, which established the ALA-BrMeTRP link; and NH-a correlated to C-4, which established the BrMeTRP-βTYR link. The above assignments of C-1 and C-8 upheld the proposed connection of N-b to C-8, of the ester oxygen to C-1, and thereby completed the definition of the macrocyclic ring. That this ring is somewhat rigid is indicated by proton vicinal J's to H-3, H-7, H-9, H-12, and H-15.

The closest marine natural product analogy to Jasplakinolide is the didemnins, which are cyclodepsipeptides comprised of a pentapeptide cyclized by a hydroxyisovalerylpropionate, and they are from the tunicate genus Tridemnum.[6] Jasplakinolide is the first cyclodepsipeptide to be isolated from a sponge. Polypeptides are only occasionally found in sponges, but they are a source of unusual amino acids.[7] The few examples of sponge polypeptides include a series of three homologous cyclic dipeptides from Tedania ignis,[8a] two related tripeptides from Clionia celata,[8b] and four related tetradecapeptides from Discodermia kiiensis[8c].

**Acknowledgement.** Research support to PC was from NOAA, National Sea Grant College Program, Department of Commerce, University of California project number R/MP-33, a grant to PC from the University Research Expeditions Program, and LVM was a Sea Grant Trainee. The U.S. Government is authorized to produce and distribute reprints for governmental purposes. We thank Dr. Hugh Webb (UCSB Mass Spectrometry Lab) for exact mass data, and PC is grateful for the cooperation of the Fiji Government.

Table 1. NMR[a] Data

| Atom # | Jasplakinolide[b] [$^{13}$C δ, APT mult] $CDCl_3$ | Jasplakinolide[b] [$^{13}$C δ, APT mult] DMSO-$d_6$ | Jasplakinolide[b] [$^1$H δ, mult = J in Hz] $CDCl_3$ | Jasplakinolide[b] [$^1$H δ, mult = J in Hz] DMSO-$d_6$ | Compound 5[c] [$^1$H δ, mult = J in Hz] $CD_3CN$ |
|---|---|---|---|---|---|
| 01 | 170.8,s | 170.1 | - | - | 3.50, dt=6.1,5.6 |
| 02 | 40.4,t | 40.8,t | 2.65, ABX<br>2.63, $J_{AB}$=15.0 | 2.90, ABX<br>2.85, $J_{AB}$=15.0 | under solvent |
| 03 | 49.2,d | 48.7,d | 5.27, bsm | 5.39, dt=8.1,8.1,4.5 | 4.99, dt=8.6,7.4,7.4 |
| 04 | 169.1,s | 168.7,s | - | - | - |
| 05 | 55.7,d | 54.5,d | 5.85, dd=9.9,6.6 | 5.79, t=6.8 | 5.45, dd=11.5,4.4 |
| 06 | 174.7,s | 172.3,s | - | - | - |
| 07 | 46.1,d | 44.5,d | 4.74, bm | 4.75, pt=7.1 | 4.36, pt=5.9 |
| 08 | 175.3,s | 174.2,s | - | - | - |
| 09 | 40.0,d | 37.9,d | 2.50, bm | 2.77, bm | 2.38, m, overlaps H-13 |
| 10 | 41.1,t | 42.0,t | 2.39, brd<br>1.89, brd $J_{AB}$=15.6 | 3.00, brd=15.3<br>2.00, dd=15.3,9.6 | 2.16, m<br>1.81, dd, near solvent |
| 11 | 133.8,s | 132.0,s | - | - | - |
| 12 | 128.3,d | 129.3,d | 4.74, bm | 4.99, d=9.0 | 4.89, d=10.3 |
| 13 | 29.3,d | 28.9,d | 2.28, bsm | 2.49, bsm | 2.41, m, overlaps H-9 |
| 14 | 43.7,t | 42.5,t | 1.30, bm<br>1.12, bm | 1.73, m<br>1.48, dt=13.2,6.6,6.6 | 1.25, m<br>1.33, m |
| 15 | 70.7,d | 69.7,d | 4.62, bsm | 4.85, sx=6.3 | 3.61, sp=5.9 |
| 16 | 131.7,s | 131.7,s | - | - | - |
| 17 | 127.4,d | 127.3,d | 6.93, d=8.4 | 7.26, d=8.5 | 7.12, d=8.8 |
| 18 | 115.7,d | 115.0,d | 6.70, d=8.4 | 6.88, d=8.5 | 6.72, d=8.8 |
| 19 | 155.9,s | 156.3,s | - | - | - |
| 20 | 23.4,t | 24.3,t | 3.37, ABX<br>3.24, $J_{AB}$=15.5 | 3.20, m | 3.37, dd=14.7,4.7<br>3.01, dd=14.7,4.1 |
| 21 | 110.4,s | 109.6,s | - | - | - |
| 22 | 127.5,s | 127.1,s | - | - | - |
| 23 | 118.3,d | 118.4,d | 7.53, d=6.9 | 7.84, d=8.4 | 7.49, d=7.7 |
| 24 | 120.4,d | 119.0,d | 7.09, t=7 | 7.19, t=7 | 7.01, t=7.7 |
| 25 | 122.5,d | 121.5,d | 7.11, t=7 | 7.28, t=7 | 7.09, t=7.7 |
| 26 | 110.6,d | 110.6,d | 7.21, d=7 | 7.45, d=8.1 | 7.28, d=7.7 |
| 27 | 136.5,s | 136.2,s | - | - | - |
| 28 | 109.2,s | 109.2,s | - | - | - |
| 29 | 17.9,q | 17.5,q | 0.66, d=6.6 | 0.79, d=6.6 | 0.69, d=5.9 |
| 30 | 20.4,q | 19.7,q | 1.11, d=6.6 | 1.15, d=6.3 | 0.91, d=5.9 |
| 31 | 18.5,q | 17.6,q | 1.55, s | 1.73, s | 1.52, s |
| 32 | 21.9,q | 21.6,q | 0.80, d=6.3 | 1.03, d=6.3 | 0.88, d=5.9 |
| 33 | 19.2,q | 19.2,q | 1.05, d=6.0 | 1.26, d=6.3 | 1.03, d=5.9 |
| 34 | 30.9,q | 30.6,q | 2.95, s | 3.18, s | 3.05, s |
| a | - | - | 7.67, d=8.4 | 8.41, d=8.5 | 7.34, d=7.9 |
| b | - | - | 6.70, d=7.7 | 7.80, d=9.0 | 6.65, d=4.6 |
| c | - | - | 9.2, bs | 11.5, bs | 9.45, bs |
| OH | - | - | 7.78, s | 9.2, bs | 7.0, s<br>3.15, t=5.6<br>2.74, d=5.6 |

[a]Chemical Shifts versus TMS for $^1$H at 300 MHz and $^{13}$C at 75 MHz. bsm = broad symmetrical multiplet.

[b]Assignments based upon $^1$H-$^1$H and $^1$H-$^{13}$C (J=140 Hz, and J=9 Hz) COSY results.

[c]Assignments based upon $^1$H-$^1$H COSY results.

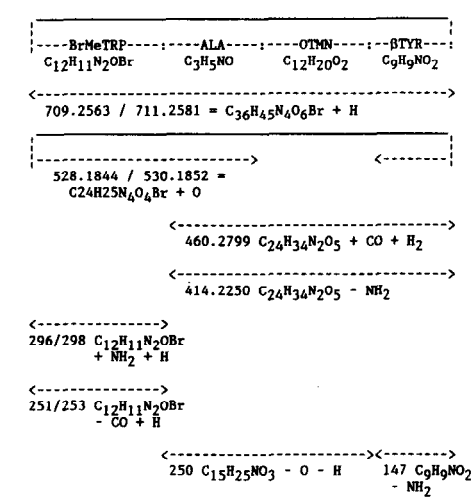
Table 2. Selected NMR COSY Results for Jasplakinolide

| $^1$H-$^1$H ($CDCl_3$) Regular | $^1$H-$^1$H ($CDCl_3$) Long Range[d] | $^{13}$C-$^1$H (DMSO-$d_6$) Long Range[e] |
|---|---|---|
| H-3 <--> H-a | | A. Carbonyls |
| H-3 <--> H-2-2' | | C-1 <--> H-2-2' |
| H-5 <--> H-20-20' | | C-4 <--> H-a |
| H-7 <--> H-b, Me-29 | | C-6 <--> Me-29, Me-34 |
| H-9 <--> Me-30 | | C-8 <--> H-10', Me-30 |
| H-10' <--> H-10, H-9 | H-10 <--> Me-31 | |
| H-12 <--> H-13 | H-10' <--> H-12 | B. BrMeTRP |
| H-13 <--> Me-32 | H-12 <--> Me-31 | C-5, C-21, C-28 <--> H-20-20' |
| H-13 <--> H-14,-14' | | C-22, C-27, C-28 <--> H-c |
| H-14-14' <--> H-15 | | C-25 <--> H-26 |
| H-15 <--> Me-33 | | C-27 <--> H-23 |
| | | C. OTMN |
| | | C-12 <--> Me-31 |
| | | C-15 <--> Me-33 |
| | | D. βTYR |
| | | C-16 <--> H-18 |
| | | C-19 <--> H-17 |

[d]Observed in the normal homo COSY spectrum

[e]Although most $^2J_{CH}$ and $^3J_{CH}$ are approximately 5 Hz, we routinely employ a J = 9 Hz value for long range COSY experiments because of increased signal to noise (due to shorter delays during $t_m$) and because the observations of correlations are not diminished when this slightly larger value of the coupling constant is employed.

Scheme 1. CI (Methane) Mass Spectrum of Jasplakinolide



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2. Identification was carried out by Dr. G. J. Bakus (USC). This sponge was first thought to be in the genus Jasplakina, but this was revised after taxonomic reexamination which was prompted by comparisons of our under-water photographs to that of an orange Jaspis also collected in Fiji by Dr. Ireland (see Ref. 5).

3. Bioassay data was kindly provided by Dr. T. Matthews at Syntex Research.

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5. At this point we also learned (personal communication) that groups led by Dr. Ireland (U. of Utah), and Dr. Faulkner (UCSD) had encountered this same natural product, and in collaboration with Dr. Clardy and co-workers (Cornell) they were able to assign substructure **B** to the tyrosine by X-ray analysis on a derivative of **1**. Work in progress, subsequently, allowed us to resolve that point in our structure proof based on chemical evidence.

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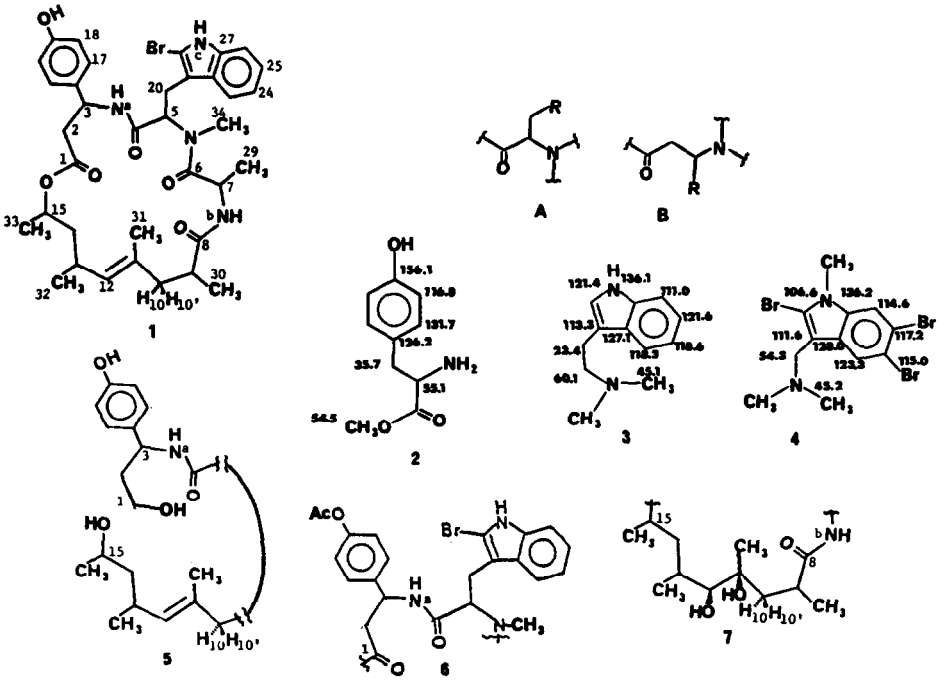

(Received in USA 24 February 1986)