MACROLIDE ANTIBIOTICS AND INDOLE ALKALOID PRODUCED FROM MARINE AND TERRESTRIAL BACTERIA

Hnin Yu Win[1], Hartmut Laatsch[2]

Abstract

Chemical investigation of terrestrial bacteria *Bacillus subtilis* M 8 and marine-derived *Streptomyces* sp. B 8406 led to the identification of macrolide antibiotics, namely, macrolactin F (1) and borrelidin (3) respectively. Additionally, the strains delivered the precursor of indole alkaloid, tryptophan (2) and indole alkaloid, 6-prenyltryptophol (4) respectively. The structures of these metabolites were elucidated based on 1D and 2D NMR experiments and mass studies. Macrolactin F (1) showed weak antibacterial properties. Borrelidin (3) showed high activity against *Escherichia coli*, *Candida albicans* and *Mucor miehei* (Tü 284) by causing inhibition zones of 25, 30 and 30 mm, at 40 μg/ disk; it showed medium activity against *Staphylococcus aureus*, *Streptomyces viridochromogenes* (Tü 57) and weak activity against *Bacillus subtilis*. It was also found to have an activity against *Artemia salina* of 93 %. Moreover, biogenesis of borrelidin (3) was discussed.

**Keywords:** macrolide, indole, alkaloid, antibiotics

Introduction

Natural products are still an important source of new pharmaceutical compounds, and natural products are metabolites from microorganisms, plants or animals (Baker *et al*., 2000). Traditionally, the major sources of these secondary metabolites are plants and terrestrial microorganisms (Barry *et al*., 1997). Over the past decades, microorganisms and especially marine bacteria have been recognized as an important source for novel bioactive compounds that are active against antibiotic-resistant human pathogens. The study of biologically active marine natural products has greatly influenced the drug discovery from natural sources in fields ranging from pharmacology to cancer medicine.

The macrolides are a class of natural products that consist of a large macrocyclic lactone ring. A great number of new macrolides from marine organisms has been discovered. Most of them have been found to have interesting biological activity such as antibiotics and some of them play vital roles as potential molecules for drug development or as tools for basic biological research. Actinomycetes are the largest source of natural macrolides and produce more than three hundred 16-membered derivatives thereof, nearly one hundred 14-memberedmacrolides and some other compounds with various ring sizes up to a 60-membered ring, including polyene macrolides,macrodiolides, macrotetrolides, and immune-suppressive macrolide lactams (Shiomi and Ōmura, 2002). Erythromycin ([McGuire] *et al*., 1952), a representative glycosidic 14-membered ring macrolide, and its related macrolides are also produced by actinomycetes and are widely used as antibacterial antibiotics.

In the search for novel and pharmacologically active metabolites, a screening of extracts from terrestrial and marine-derived bacteria was performed. In the screening of *Bacillus subtilis* M 8, the strain delivered macrolactin F (1) and tryptophan (2). Marine *Streptomyces* sp. B 8406 delivered borrelidin (3) and 6-prenyltryptophol (4).

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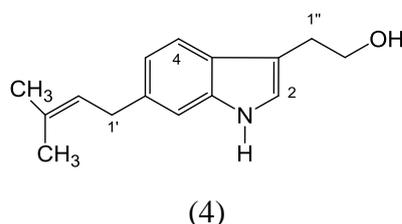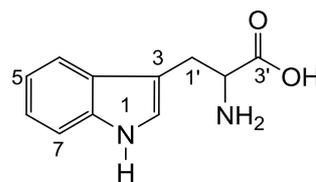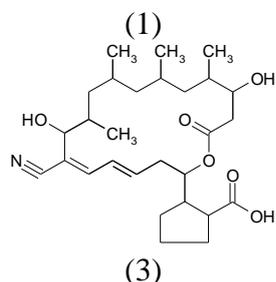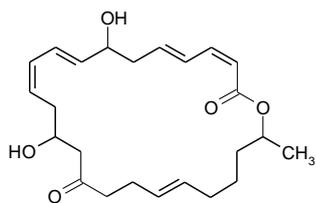
Materials and Methods

General Experimental Procedures

NMR spectra were measured on a Varian Inova 600 (599.740 MHz) and a Varian Unity 300 (300.145 MHz) spectrometer. ESIMS were measured on a Quattro Triple Quadrupol mass spectrometer with a Finnigan TSQ 7000 with nano-ESI API ion source. EIMS at 70 eV with Varian MAT 731, Varian 311A, AMD-402, high resolution with perflurokerosene as standard. HRESIMS were measured on a Micromass LCT mass spectrometer coupled with a HP 1100 HPLC and a diode array detector. Column chromatography was carried out on MN silica gel 60, 0.05-0.2 mm; TLC was performed on Polygram SIL G/UV$_{254}$. All silica gel materials were purchased from Macherey-Nagel, Düren, Germany. Size exclusion chromatography was done on Sephadex LH-20 (Lipophilic Sephadex; Amersham Biosciences, Freiburg, Germany, purchased from Sigma-Aldrich Chemie, Steinheim, Germany). XAD-16 adsorber resin was obtained from Rohm and Haas (Frankfurt, Germany).

Spray reagents

Anisaldehyde/sulphuric acid: 1 mL anisaldehyde was added to 100 mL of a stock solution containing 85 mL methanol, 14 mL acetic acid and 1 mL sulphuric acid. After spraying, the TLC cards were heated with hot air until colour development.

Biological screening

The crude extract was dissolved in CHCl$_3$/10% MeOH (400 μg/paper disk), in which the paper disks were dipped, dried under sterile conditions (flow box) and put on an agar plates inoculated with *Bacillus subtilis* (ATCC6051), *Staphylococcus aureus*, *Streptomyces viridochromogenes* (Tü 57), *Escherichia coli*, *Chlorella vulgaris*, *Chlorella sorokiniana*, *Scenedesmus subspicatus*, *Candida albicans* and *Mucor miehei* (Tü 284). The plates were incubated at 37 °C for bacteria (12 h), 27 °C for fungi (24 h), and 24-26 °C under day-light for micro-algae (96 h). The diameter of the inhibition zones was measured by ruler.

Brine shrimp microwell cytotoxicity assay

To a 500 mL separating funnel filled with 400 mL of artificial seawater, 0.5 g of dried eggs of *Artemia salina* was added. The suspension was aerated by bubbling air into the funnel and kept for 24 to 48 h at room temperature. After aeration had been removed, the suspension was kept for 1 h undisturbed, whereby the remaining unhatched eggs dropped. In order to get a higher density of larvae, one side of the separating funnel was covered with aluminium foil and the other illuminated with a lamp, whereby the phototropic larvae were gathering at the illuminated side and could be collected by pipette. 30 to 40 shrimp larvae were transferred to a deep-well microtiter plate (wells diameter 1.8 cm, depth 2 cm) filled with 0.2 mL of salt water and the dead larvae counted (number N). A solution of 20 µg of the crude extract in 5 to 10 µL DMSO was added and the plate kept at room temperature in the dark. After 24 h, the dead larvae were counted in each well under the microscope (number A). The still living larvae were killed by addition of ca. 0.5 mL methanol so that subsequently the total number of the animals could be determined (number G). The mortality rate M was calculated in %. Each test row was accompanied by a blind sample with pure DMSO (number B) and a control sample with 1 µg/test actinomycin D. The mortality rate M was calculated using the following formula:

$$M = \left[\frac{(A - B - N)}{(G - N)} \right] \cdot 100$$

Where

M	=	percent of the dead larvae after 24 h
A	=	number of the dead larvae after 24 h
B	=	average number of the dead larvae in the blind samples after 24 h
N	=	number of the dead larvae before starting of the test and
G	=	total number of brine shrimp

Primary screening

Antibiotic screening (disk diffusion test): The test is performed using paper discs with a diameter of 8 mm under standardized conditions. If the inhibition zone is ranging from 11 to 20 mm, the compound is considered to be weakly active (+), from 21 to 30 mm designated as active (++) and over 30 mm is highly active (+++). Chemical screening: evaluation of the separated bands by the number, intensity and colour reactions with different staining reagents on TLC. Toxicity test with brine shrimps: by counting survivors after 24 h, the mortality of the extracts was calculated (see above). The extracts, fractions or isolated compounds were considered inactive when the mortality rate was lower than 10% (-), from 10 to 59% as weakly active (+), from 60 to 95% as active (++) and over 95% as strongly active (+++).

Fermentation, Work-up and Isolation

The strain *Bacillus subtilis* M 8 was cultivated on LB-medium [tryptone (10 g), yeast extract (5 g), sodium chloride (10 g) in 1000 mL tap water] in a 25 L scale. Well-grown agar plates were used to inoculate 60 Erlenmeyer flasks (1 L flask size), each containing 250 mL LB medium at pH 7. The cultures were cultivated on the linear shaker for 72 h at 34 °C. The resulting culture broth was filtered with the aid of the filter press. The water phase was subjected

to an XAD-16 column and extracted with MeOH. The mycelium was extracted with ethyl acetate and acetone. The two combined phases was brought to dryness under reduced pressure to yield 14.2 g crude extract. The resulting crude extract was subjected to a silica gel column chromatography using a CH_2Cl_2/MeOH gradient and separated into fractions I-V. Further purification of fractions III by Sephadex LH-20 and RP-18 silica gel yielded macrolactin F (1). Tryptophan (2) was isolated as white amorphous from the very polar fraction. It exhibited UV absorption at 254 nm and gave red colour reaction with anisaldehyde/sulphuric acid.

The subculture of marine *Streptomyces* sp. B 8406 was used to inoculate a 25 L shaker culture using M_2^+ medium [malt extract (10 g), yeast extract (4 g) and glucose (4 g) were dissolved in 500 mL tap water and 500 mL sea water, pH 7.8 before sterilisation]. After 7 days, the fermentor broth was harvested and the resulting reddish brown culture broth was subjected to filtration over Celite using a filter press. The filtrate was given on XAD-16, and the adsorbed metabolites were eluted with methanol. The methanol was evaporated under reduced pressure and the resulting water residue was extracted by ethyl acetate. The biomass was extracted by ethyl acetate and acetone until the colour had disappeared. The combined organic solutions were evaporated under vacuum to yield 2.56 g of reddish brown crude extract. Separation was performed by a flash silica gel column chromatography (CH_2Cl_2/MeOH gradient, 2.5 L CH_2Cl_2, 1.0 L CH_2Cl_2/2% MeOH, 1.0 L CH_2Cl_2/4% MeOH, 1.0 L CH_2Cl_2/5% MeOH, 0.5 L CH_2Cl_2/10% MeOH, 0.5 L CH_2Cl_2/20% MeOH, 0.5 L CH_2Cl_2/50% MeOH, 0.5 L MeOH). Borrelidin (3) (10 mg) was isolated from fraction III by subjecting on a silica gel column with a CH_2Cl_2:MeOH gradient. 6-prenyltryptophol (4) was isolated from fraction II by passing it over Sephadex LH-20 (MeOH).

Results and Discussion

Macrolactin F (1)

Macrolactin F (1) was isolated as oily substance and showed a UV absorbing band at 254 nm, which stained to green with anisaldehyde-sulphuric acid. Structure assignment of macrolactin F was achieved by interpretation of spectroscopic analysis, particularly by H,H COSY NMR data. In the ^{1}H NMR spectrum (Figure 1), there were 10 olefinic proton signals, three oxygen-bound proton signals, many CH_2 signals and one methyl doublet. Analysis of the ^{13}C NMR (Figure 2) and HSQC spectra showed a ketone and an ester carbonyl signal at δ 211.8 and 167.9, ten olefinic carbon signals, three oxygenated carbon signals, 8 CH_2 and one CH_3. The (+)-ESI mass spectrum indicated an $[M + Na]^+$ ion peak at *m/z* 425. By analysis of the COSY spectrum (Figure 3), three substructures were constructed (Figure 4).
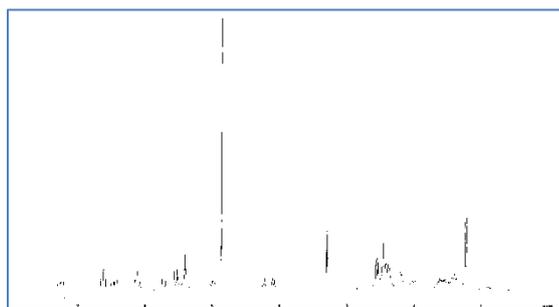

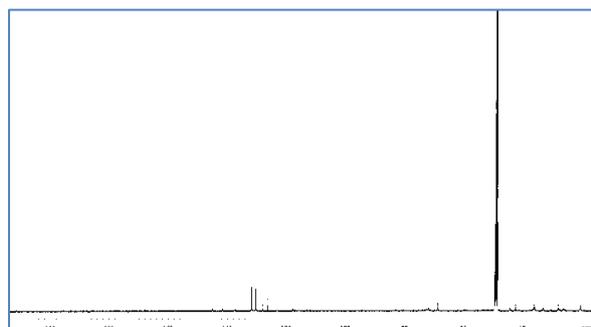**Figure 1** ^{1}H NMR spectrum (CD$_3$OD, 300 MHz) of macrolactin F

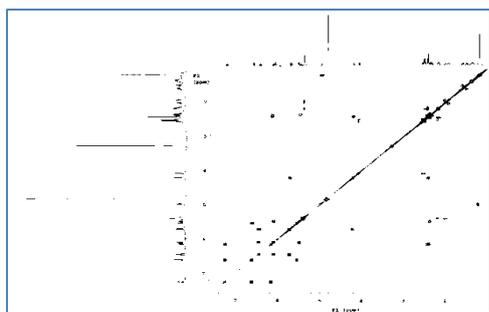**Figure 2** ^{13}C NMR spectrum (CD$_3$OD, 125 MHz) of macrolactin F

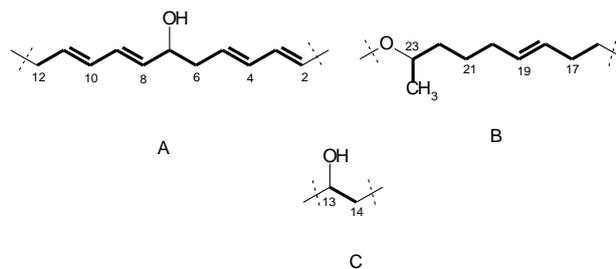**Figure 3** COSY spectrum (CD₃OD, 600 MHz) of macrolactin F

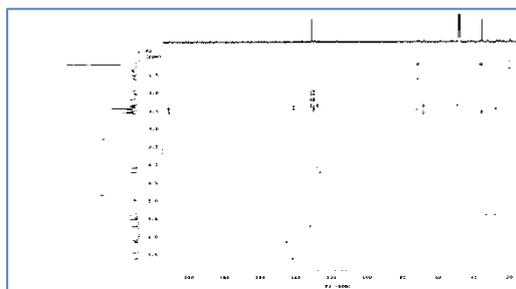**Figure 4** Fragments of macrolactin F (1) resulting from the COSY (━) spectrum

Figure 5 HMBC spectrum (CD₃OD, 600 MHz) of macrolactin F

While the methine proton at δ 5.55 (H-2) showed the HMBC correlation to the acid carbonyl at δ 167.9 (C-1), the two CH₂ groups at δ 2.56 and 2.46 (H-14 and H-16) showed HMBC interactions with the ketone carbonyl at δ 211.8 (C-15) (Figure 5). The H-14 exhibited correlation again with one oxygenated methine carbon at δ 68.7 (C-13) and methylene group at δ 35.9 (C-12) from fragment A. The HMBC correlations of a methyl doublet at δ 1.23 (H-24) with one oxygenated methine carbon at δ 71.8 (C-23) and one methylene at δ 36.2 (C-22) confirmed the COSY correlations (Figure 6).
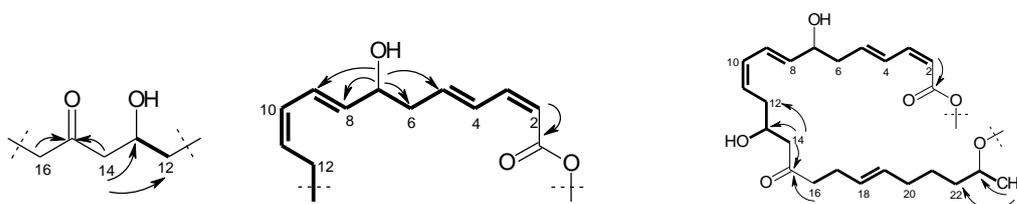

Figure 6 Selected HMBC ($\rightarrow$) correlations in macrolactin F

By closing the two open chains as lactone, the complete structure was deduced and it was assigned as macrolactin F. It was a geometrical isomer of macrolactin K, which has a *trans* double bond between C-10 and C-11 (Figure 7). The coupling constant between H-10 and H-11 was less than 12 Hz in compound and it was interpreted as *cis* double bond.
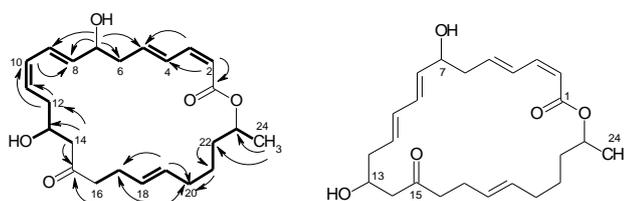

Figure 7 H,H COSY (━)and selected HMBC ($\rightarrow$) correlations of macrolactin F (1) and macrolactin K

Table 1 shows the NMR spectroscopic data for macrolactin F (1).

Table 1 NMR spectroscopic data for macrolactin F (1)

Position	δ_C	δ_H (mult.; *J* in Hz)
1	167.9	CO
2	117.9	5.55 (d, 11.4)
3	145.1	6.62 (t, 11.4)
4	130.4	7.26 (ddd, 1.1, 11.3, 15.3)
5	141.6	6.17 (dd, 8.1, 14.6)
6	42.4	2.46 (t, 7.4)
7	72.6	4.23 (q, 6.4)
8	137.5	5.73 (dd, 6.4, 15.3)
9	126.5	6.46 (dd, 10.9, 15.2)
10	131.8	6.11 (t, 11.0)
11	128.1	5.49 (q, 8.1)
12	35.9	2.39 (m)
13	68.7	4.09 (q, 6.3)
14	49.6	2.56 (dd, 4.5, 6.2)
15	211.8	CO
16	44.4	2.46 (t, 7.4)
17	27.9	2.21 (m)
18	130.3	5.41 (m)
19	131.9	5.41 (m)
20	32.9	2.05, 1.96 (m)
21	26.1	1.39 (m)
22	36.2	1.62, 1.53 (m)
23	71.8	4.99 (m)
24	20.3	1.23 (d, 6.3)

Tryptophan (2)

The ^{1}H NMR spectrum (Figure 8) of tryptophan (2) shows two 1H doublets and two 1H triplets of doublets at δ 7.59, 7.37, 7.06 and 6.97 for a 1,2-distubstituted benzene ring. Additionally, the spectrum shows a singlet at δ 7.29. The signal pattern in the aromatic region indicated the presence of a 3-substituted indolic moiety. In the aliphatic region, a heteroatom bearing methine signal at δ 3.61 and diastereotopic methylene signals at δ 3.36 and 3.07 indicated the neighbourhood of an sp^2 carbon or heteroatom.
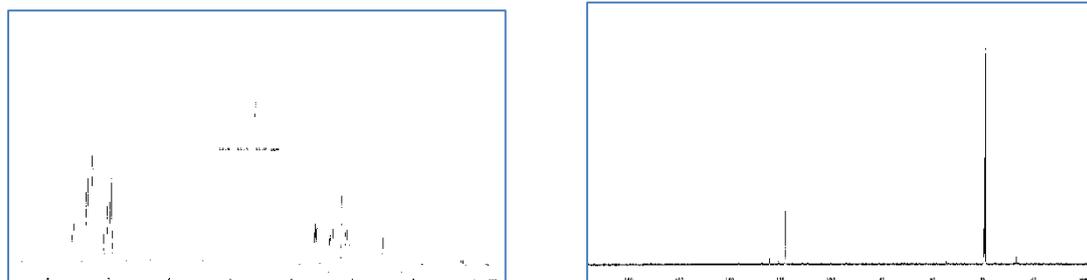

Figure 8 ^{1}H NMR spectrum (CD$_3$OD, 300 MHz) and ^{13}C NMR spectrum (CD$_3$OD, 125 MHz) of tryptophan

The ^{13}C NMR spectrum shows 11 signals, whereof those at δ 171.2 could be assigned to carbonyl of acid, ester or amide. In addition, there were eight signals sp^2 carbons, one nitrogen bearing carbon and one methylene carbon.

The (-)-ESI mass spectrum indicated a *quasi*molecular ion at *m/z* 203 [M - H]⁻ leading to the molecular weight of 204 Dalton. Comparison with authentic spectra identified the isolated compound as tryptophan. Tryptophan is one of the 20 proteinogenic as well as an essential amino acid. In genetic code, it is encoded by the codon UGG.

(2)
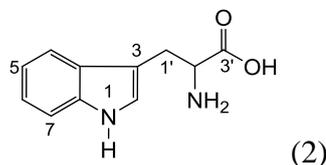
Borrelidin (3)

Borrelidin (3) was isolated as colourless oil from fraction III by passing through a silica gel column. It showed UV absorption at 254 nm and stained to green with anisaldehyde/sulphuric acid. The ESI mass spectrum indicated an [M + Na]⁺ ion peak at *m/z* 512 for a molecular weight of 489 Dalton, and the HR ESI mass spectrum afforded the molecular formula $C_{28}H_{43}NO_6$ with 8 double bond equivalents.

The ^1H NMR spectrum displayed three olefinic protons at δ6.89, 6.59 and 6.30, three oxygenated protons at δ 4.97, 4.18 and 3.92, one methyl doublet at δ1.01, three overlapped methyl doublets at δ 0.83 and the other multiplets between δ 2.80 and 1.01, which were assigned as long chain of CH and CH₂ groups. The carbon spectrum indicated the presence of 28 carbons, which were assigned by chemical shifts and analysis of HSQC to two carbonyls of acid, ester or amide, three methine sp^2 atoms, two olefinic quaternary atoms, three oxygenated methine atoms, eight aliphatic methylenes, six aliphatic methines and four methyls. $^1H,^1H$ COSY data established the fragments **A, B, C** and **D** (Figure 9-13).
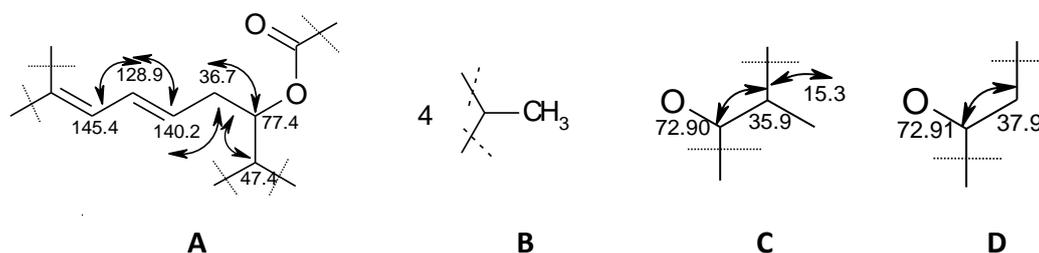

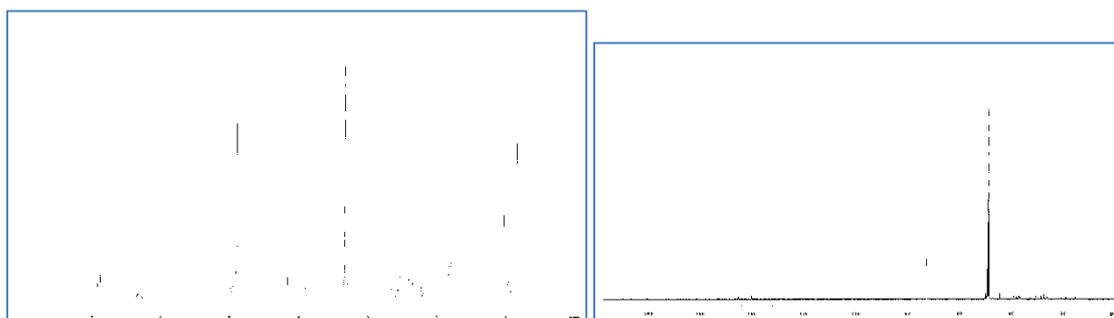**Figure 9** Partial structures of borrelidin (3) from H,H COSY spectrum

Figure 10 ^1H NMR spectrum (CD₃OD, 300 MHz) Figure 11 ^{13}C NMR spectrum (CD₃OD,
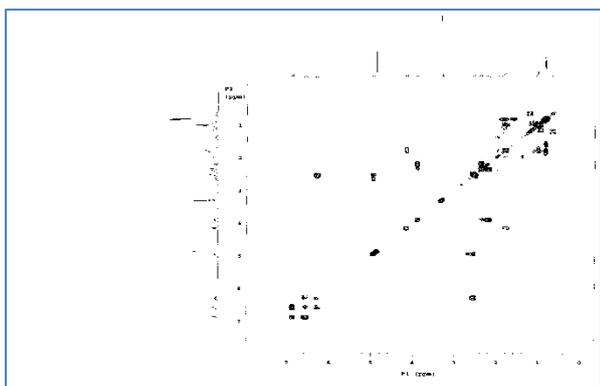   of borrelidin (3) 125 MHz) of borrelidin (3)

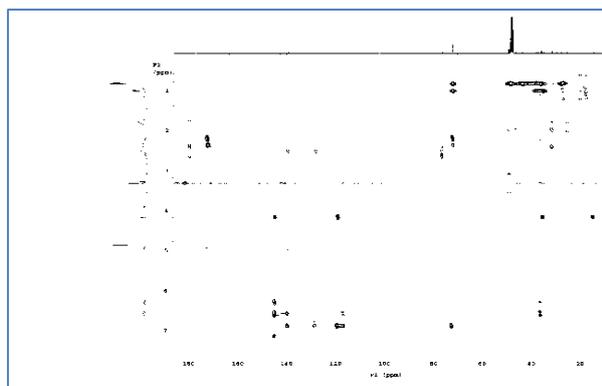**Figure 12** H, H COSY spectrum (CD₃OD, 600 MHz) of borrelidin (3)

Figure 13 HMBC spectrum (CD₃OD, 600 MHz) of borrelidin (3)

The HMBC data were used to connect these partial structures and to obtain the complete structure. In the HMBC spectrum (Figure 13), from H-6 (δ 6.80, δ 145.4) correlations were observed to C-4, C-5, C-7, C-8 (fragment C) and C-24. There was only one position possible for the nitrogen, which was attached to C-24. Hence, it was assigned as nitrile group, which was confirmed by the chemical shift (Figure 14).
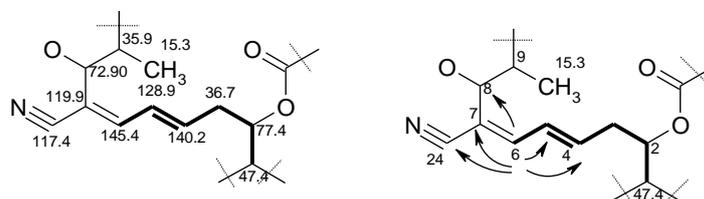

Figure 14 H,H COSY (━) and HMBC (→) correlations of borrelidin (3)

The methyl doublet at δ1.01 (δ_C 15.3) correlated to C-8, C-9 and C-10 (CH₂, δ 39.1), while the C-10 methylene signal showed correlation to the methine carbon at δ27.6 (C-11), which was connected with the methyl group at δ20.9 (C-25). This methyl correlated with the methylene at δ 49.9 (C-12) and the methylene carbons at δ 49.9 (C-12) and 44.6 (C-14) correlated to three methyl doublets at δ0.83 (Figure 15).
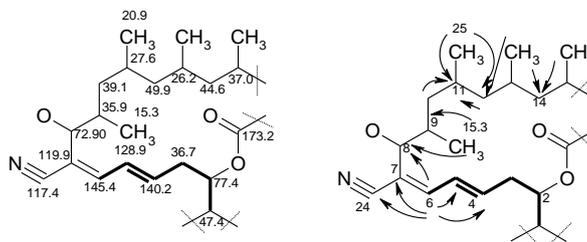

Figure 15 HMBC (→) correlations of borrelidin (3)

H-2 (δ 4.99, δ 77.4) and H-17 (δ 2.35, δ 37.9, fragment D) showed correlations to carbonyl at δ 173.2 and the methine proton at δ 3.90 (δ 72.91) correlated to the methyl at δ 18.7. According to these data, a macrolide ring system could be drawn (Figure 16).
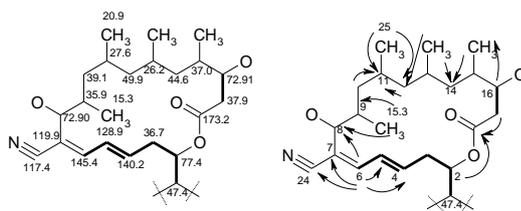
Figure 16 HMBC (→) correlations of borrelidin (3)

The methine protons at δ 2.66 (δ 47.4, C-19) and 2.40 (δ 50.2, C-20) showed cross peaks to carbonyl at δ 180.6. The three remaining CH_2 groups were assigned as a ring system, which accounted for the remaining double bond equivalents. The isolated compound was assigned as nitrile-containing macrolide antibiotic borrelidin (3) (Figure 17).
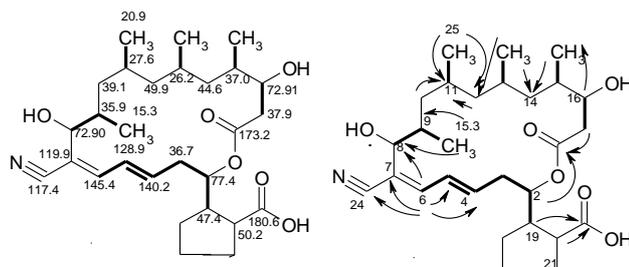

Figure 17 H,H COSY (—)and HMBC (→) correlations of borrelidin (3)

In our agar diffusion test, borrelidin showed high activity against *Escherichia coli*, *Candida albicans* and *Mucor miehei* (Tü 284) by causing inhibition zones of 25, 30 and 30 mm at 40 μg/ disk; it showed medium activity against *Staphylococcus aureus*, *Streptomyces viridochromogenes* (Tü 57) and weak activity against *Bacillus subtilis*. It was also found to have an activity against *Artemia salina* of 93 %.

Biogenesis of Borrelidin (3)

Macrolide antibiotics are synthesized by a series of condensation reactions catalysed by polyketide synthetases (PKSs). Various post-polyketide modifications involved mainly methylations, hydroxylations, epoxidation and glycosylation. Borrelidin contains a non-glycosylated, macrocyclic polyketide lactone ring and presents a unique structural feature not frequently occurring in any other macrolide natural products; that is the presence of a nitrile moiety at C-7 and a 1,2- *trans* substituted cyclopentane carboxylic acid moiety attached to C-2. The production of nitrile-containing compounds by microorganisms is relatively rare, even though more than 120 nitrile-containing natural products from different sources have been reported. The biosynthesis of borrelidin was predicted to involve a borrelidin polyketide skeleton which must be performed by a modular polyketide synthase (PKS) incorporated with *trans* cyclopentane-1,2-dicarboxylic acid as the starter unit and eight extender units (three malonyl-CoA and five methylmalonyl-CoA units) in the pre-polyketide step (Figure 18) ([Olano] *et al.*, 2004). In the post-polyketide step, the formation of the nitrile group on C-7 from methyl group, which is formed from third round chain extension of methyl malonyl CoA, it was proposed that C-7 methyl carbon was first oxidized by BorI to introduce an allylic hydroxyl group and then to formyl derivative. Conversion of the formyl group to an amino group was performed by aminotransferase BorJ and further oxidation catalysed by BorI to a gem-dihydroxyl species and then by subsequent dehydration to borrelidin (Figure 19). BorI and BorJ play a major role in the

biosynthesis of borrelidin, therefore inactivation of these genes would cause the accumulation of pre-borrelidin.
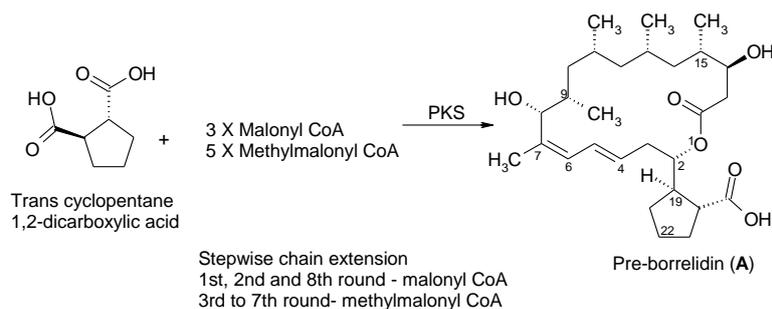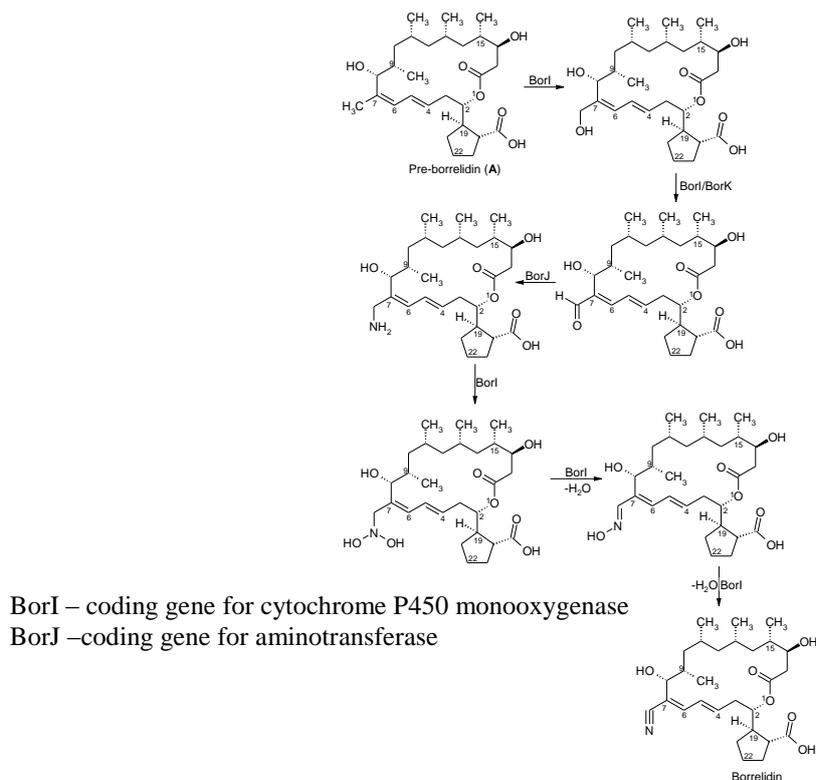

Figure 18 Proposed mechanism for pre-borrelidin (A)

BorI – coding gene for cytochrome P450 monooxygenase
BorJ –coding gene for aminotransferase

Figure 19 Proposed mechanism for the generation of the nitrile moiety as post-polyketide step in borrelidin synthesis (Olano, et al., 2004)

6-Prenyltryptophol (4)

   The ^{1}H NMR spectrum (Figure 20) of 6-prenyltryptophol (4) shows the presence of two *ortho* coupled protons at δ 7.41 and 6.82 and two singlets at δ 7.09 and 6.98. These data suggested the presence of a 3,6-disubsituted indole nucleus. In the aliphatic region, the spectrum exhibited two triplets at δ 3.78 (^3J = 7.2 Hz, 2H) and 2.93 (^3J = 7.2 Hz, 2H) for a 1, 2-disubstituted ethanediyl group connected with two electronically different groups namely an sp^2 carbon and a hetero atom (O or N). The spectrum showed additionally a singlet at δ 1.74 for two CH$_3$ groups attached to an sp^2 carbon. The spectrum also revealed that an sp^2 attached proton (1H triplet at δ 5.35, ^3J = 7.4 Hz) must be of adjacent to a methylene group. This allylic methylene group showed a doublet at δ 3.39 (2 H, ^3J = 6.8 Hz). These patterns are evidence of an isoprene unit attached at C-6 position of the indole ring.
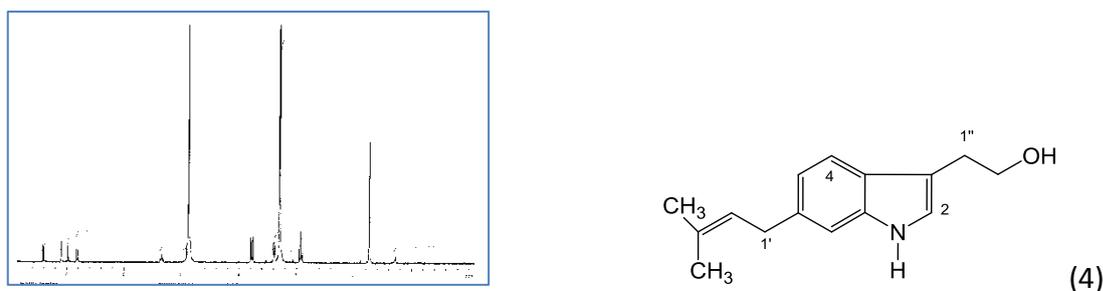
Figure 20 ^{1}H NMR spectrum (CD$_3$OD, 300 MHz) of 6-prenyltryptophol

The EI mass spectrum showed the molecular ion peak at *m/z* 229. It gave the base peak at *m/z* 198 by the loss of CH$_2$OH (MW 31) from the molecular ion. The compound was identified as 6-prenyltryptophol by searching in AntiBase with the above spectroscopic data as well as the molecular weight. It was further confirmed by comparison with an authentic spectrum and literature data. 6-Prenyltryptophol showed activities against fungi and was cytotoxic against a panel of 14 different tumor cell lines with the GI$_{50}$ values in a micromolar range (José *et. al.*, 2003)

Table 2 Antimicrobial Activity of the Crude Extract from Strain B 8406 on M$_2$$^+$ Medium [40 µL/paper disk (100 mg/mL)], Diameter of Inhibition Zones in mm.

Test microorganisms	Inhibition zone ∅ [mm]
Bacillus subtilis	30
Staphylococcus aureus	19
Streptomyces viridochromogenes (Tü 57)	12
Escherichia coli	11
Candida albicans	18
Chlorella vulgaris	11

Macrolactin F (1): oily substance, 5.1 mg, UV absorbing band at 254 nm, R_f = 0.11 (CH$_2$Cl$_2$/10% MeOH), green with anisaldehyde/sulphuric acid. – ^{1}**H NMR** (CD$_3$OD, 300 MHz) and – ^{13}**C NMR** (CD$_3$OD, 125 MHz), (Table 1). – **(+)-ESIMS** 425 ([M + Na]$^+$, 86), 827 ([2 M + Na]$^+$, 100).

Tryptophan (2): white amorphous, 30 mg, R_f = 0.35 (CHCl$_3$/5% MeOH), polar UV absorbing band at 254 nm, red colour with anisaldehyde/sulphuric acid. – ^{1}**H NMR** (CD$_3$OD, 300 MHz) δ 7.59 (d, ^3J = 7.8 Hz, 1H, H-4), 7.37 (d, ^3J = 8.0 Hz, 1H, H-7), 7.29 (s, 1H, H-2), 7.06 (td, ^3J = 7.1 Hz, ^4J = 1.1 Hz, 1H, H-5), 6.97 (td, ^3J = 7.9 Hz, ^4J = 0.9 Hz, 1H, H-6), 3.61 (dd, ^3J = 4.3 Hz, 1H, H-2'), 3.36 (dd, ^3J = 15.1 Hz, ^4J = 4.2 Hz, 1H, H$_A$-1'), 3.07 (dd, ^3J = 8.4 Hz, 1H, H$_B$-1'). – **(+)-ESIMS** *m/z* 205 ([M + H]$^+$, 100). – **(-)-ESIMS** *m/z* 203 ([M - H]$^-$, 100), 407 ([2 M - H]$^-$, 60).

Borrelidin (3): colourless oil, 10 mg, R_f = 0.90 (CH$_2$Cl$_2$/10% MeOH), very strong UV absorbing band at 254 nm, green with anisaldehyde/sulphuric acid. – ^{1}**H NMR** (CD$_3$OD, 300 MHz) δ 6.89 (d, ^3J = 11.3 Hz, 1H, H-6), 6.59 (t, ^3J = 11.4 Hz, 1H, H-5), 6.30 (m, 1H, H-4), 4.97 (dt, ^3J = 11.1 Hz, 3.2 Hz, 1H, H-2), 4.18 (d, ^3J = 9.8 Hz, 1H, H-8), 3.92 (dt, ^3J = 10.1 Hz, 2.9 Hz, 1H, H-16), 2.66 (quin, ^3J = 9.1 Hz, 1H, H-19), 2.54 (m, 2H, CH$_2$-3), 2.40 (m, 1H, H-20),

2.35 (d, ^3J = 2.8 Hz, 2H, CH$_2$-17), 1.99 (m, 2H, H$_A$-21, 23), 1.83 (m, 6H, H-9, 13, 15, H$_B$-21, CH$_2$-22), 1.62 (m, 1H, H-11), 1.36 (m, 1H, H$_B$-23), 1.20 (m, 1H, H$_A$-10), 1.18-0.88 (m, 4H, CH$_2$-12, 14), 1.01 (d, ^3J = 6.4 Hz, 3H, CH$_3$-9), 0.83 (d, ^3J = 6.8 Hz, 9H, CH$_3$-11, 13, 15), 0.68 (br t, 1H, H$_B$-10). – ^{13}**C NMR** (CD$_3$OD, 125 MHz) δ 180.6 (C-28), 173.2 (C-18), 145.4 (CH-6), 140.2 (CH-4), 128.9 (CH-5), 119.9 (Cq-7), 117.4 (C$_q$-24), 77.4 (CH-2), 72.91 (CH-16), 72.90 (CH-8), 50.2 (CH-20), 49.9 (CH$_2$-14), 47.4 (CH-19), 44.6 (CH$_2$-12), 39.1 (CH$_2$-10), 37.9 (CH$_2$-17), 37.0 (CH-15), 36.7 (CH$_2$-3), 35.9 (CH-9), 32.5 (CH$_2$-21), 30.5 (CH$_2$-22), 28.5 (CH-13), 27.6 (CH-11), 26.2 (CH$_2$-23), 20.9 (CH$_3$-27), 19.1 (CH$_3$-26), 18.7 (CH$_3$-28), 15.4 (CH$_3$-25). – **(+)-ESIMS** *m/z* 1001 ([2 M + Na]$^+$, 100), 512 ([M + Na]$^+$, 8). – **(-)-ESIMS** *m/z* 977 ([2 M - H]$^-$, 86), 488 ([M - H]$^-$, 100). – **(+)-HRESIMS** *m/z* 512.29838 [M + Na]$^+$, (calcd. 512.29826 for C$_{28}$H$_{43}$NO$_6$Na).

6-Prenyltryptophol (4): colourless solid, 2.1 mg, R_f = 0.5 (CH$_2$Cl$_2$/ 5% MeOH), UV absorption band at 254 nm, pink by spraying with anisaldehyde/sulphuric acid. -^{1}**H NMR** (CD$_3$OD, 300 MHz) δ 7.41 (d, ^3J = 7.9 Hz, 1 H, H-4), 7.09 (s, 1 H, H-7), 6.98 (s, 1 H, H-2), 6.82 (dd, ^3J = 8.1 Hz, ^4J = 1.4 Hz, 1 H, H-5), 5.35 (m, 1 H, H-2'), 3.78 (t, ^3J = 7.2 Hz, 2 H, CH$_2$-2"), 3.39 (d, ^3J = 6.8 Hz, 2 H, CH$_2$-1'), 2.93 (d, ^3J = 7.5 Hz, 2 H, CH$_2$-1"), 1.74 (s, 6 H, CH$_3$-3'). - **EIMS** (70 eV) *m/z* 229 ([M]$^{+\cdot}$, 86), 198 ([M - CH$_2$OH]$^{+\cdot}$, 100), 182 (10).

Conclusion

In this study, macrolide antibiotics macrolactin F (1) from *Bacillus subtilis* M 8 and borrelidin (3) from marine *Streptomyces* sp. B 8406 were isolated and characterized. Macrolactin F (1) showed weak antibacterial properties. Borrelidin (3) showed high activity against *Escherichia coli*, *Candida albicans* and *Mucor miehei* (Tü 284), medium activity against *Staphylococcus aureus*, *Streptomyces viridochromogenes* (Tü 57) and weak activity against *Bacillus subtilis*. It was also found to have an activity against *Artemia salina* of 93 %.

Acknowledgements

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