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Halichoblelide, a potent cytotoxic macrolide from a *Streptomyces* species separated from a marine fish

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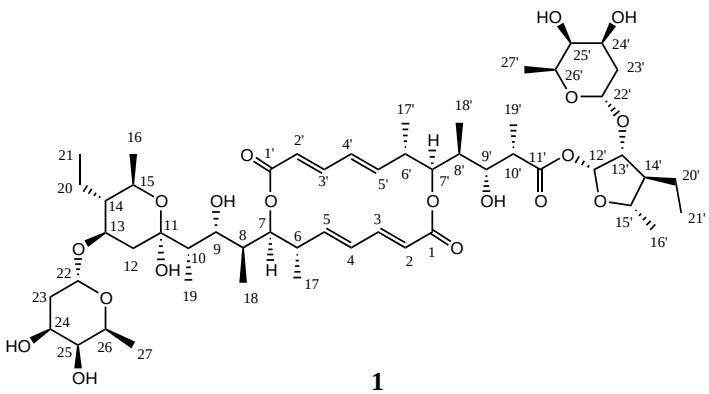
Abstract—Halichoblelide, a novel macrolide with potent cytotoxicity against tumour cells in culture, has been isolated from a strain of *Streptomyces hygroscopicus* originally separated from the marine fish *Halichoeres bleekeri*, and the absolute stereostructure has been elucidated on the basis of spectroscopic analyses using 1D and 2D NMR techniques and some chemical transformations.

Up until today some of bioactive materials from marine animals have been isolated from various bacteria. Recently we have focused our attention on new antitumour materials produced by microorganisms from marine organisms.[1] Previously we have isolated the structurally unique and potent cytotoxic macrolide, halichomycin, from a strain of *Streptomyces hygroscopicus* OUPS-N92 originally obtained from the marine fish *Halichoeres bleekeri*.[2] Further investigation for metabolites of this fungal strain has now led to the isolation of one additional new cytotoxic compound designated as halichoblelide.

The microorganism from *H. bleekeri* fish was cultured at 27°C for a week in a medium (80 l) containing 0.1% cone steep liquor and 1% dextrin in artificial seawater adjusted to pH 7.5. After incubation the AcOEt extract of the culture filtrate was purified by bioassay-directed fractionation (cytotoxicities against P388 cell line) with employing stepwise combination of Sephadex LH-20 and silica gel column chromatography followed by reversed-phase HPLC. This sequence of purification yielded 7.2 mg of halichoblelide (**1**)[3] as colourless powder.

Based on an $[M+Na]^+$ peak of its methyl acetal derivative **2** in HRSIMS,[3] the molecular formula of halichoblelide (**1**) was deduced as $C_{54}H_{85}O_{19}$. The UV and IR spectra of halichoblelide (**1**) exhibited absorption bands characteristic for an alcohol and a conjugated carbonyl group. A close inspection of the ^1H and ^{13}C NMR spectra of **1** (Table 1) by DEPT and ^1H–^{13}C correlation spectroscopy (COSY) experiments revealed the presence of two primary methyls, ten secondary methyls, five sp^3-hybridised methylenes, 25 sp^3-methines including 14 oxymethines and three anomeric methines, one anomeric quaternary sp^3-carbon, two 1,3-diene moieties, three ester carbonyls, and seven



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Keywords: halichoblelide; *Streptomyces hygroscopicus*; macrolide; marine fish.

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Table 1. NMR spectral data of halichoblelide A (**1**) in $CDCl_3$

Position	δ_H[a]	J/Hz	δ_C	Position	δ_H[a]	J/Hz	δ_C
1			168.10 (q)[b]	1′			170.02 (q)[b]
2	5.69 d	15.8	120.95 (t)	2′	5.66 d	15.8	121.42 (t)
3	6.98 dd	15.8, 11.2	145.05 (t)	3′	6.96 dd	15.8, 11.2	145.10 (t)
4	6.12 dd	14.5, 11.2	132.02 (t)	4′	6.06 dd	14.5, 11.2	131.35 (t)
5	5.67 dd	14.5, 10.3	144.19 (t)	5′	5.61 dd	14.5, 10.2	144.77 (t)
6	2.54 tq	10.3, 7.0	40.90 (t)	6′	2.49 tq	10.3, 7.0	41.66 (t)
7	4.77 dd	10.3, 1.5	77.88 (t)	7′	5.12 dd	10.3, 1.6	76.63 (t)
8	1.96 dqd	10.3, 7.1, 1.5	35.87 (t)	8′	1.91 dqd	10.3, 7.1, 1.6	35.76 (t)
9	4.12 ddd	10.3, 0.8, 2.1	70.65 (t)	9′	3.52 br d	10.3	72.98 (t)
10	1.72 qd	7.3, 0.8	41.60 (t)	10′	2.32 qd	7.0, 3.6	40.08 (t)
11			99.05 (q)	11′			158.2 (q)
12α	2.38 dd	10.2, 4.7	38.93 (s)	12′	4.78 d	3.0	95.13 (t)
12β	1.03 t	10.2		13′	4.07 dd	6.4, 3.0	68.67 (t)
13	3.97 td	10.2, 4.7	70.26 (t)	14′	1.56 tt	6.4, 5.0	44.01 (t)
14	1.19 tt	10.2, 3.8	48.42 (t)	15′	3.96 quintet	6.4	74.23 (t)
15	3.91 dq	10.2, 6.4	66.55 (t)	16′	1.29 d	6.4	18.67 (p)
16	1.11 d	6.4	19.12 (p)	17′	1.05 d	7.0	15.49 (p)
17	1.02 d	7.0	14.90 (p)	18′	0.93 d	7.1	9.51 (p)
18	0.81 d	7.1	8.75 (p)	19′	1.05 d	7.0	10.63 (p)
19	1.01 d	7.3	7.07 (p)	20′A	1.42 dqd	12.5, 7.5, 5.0	21.65 (s)
20A	1.44 dqd	12.9, 7.5, 3.8	19.39 (s)	20′B	1.50 dqd	12.5, 7.5, 5.0	
20B	1.62 dqd	12.9, 7.5, 3.8		21′	0.89 t	7.5	9.75 (p)
21	0.86 t	7.5	9.10 (p)	22′	5.10 t	2.8	93.22 (t)
22	5.05 t	2.1	94.25 (t)	23′α	1.82 dt	12.3, 2.8	33.33 (s)
23α	1.79 dt	12.3, 2.1	33.58 (s)	23′β	1.83 ddd	12.3, 10.8, 2.8	
23β	1.81 ddd	12.3, 11.1, 2.1		24′	3.98 br d	10.8	66.02 (t)
24	3.97 br d	11.1	66.07 (t)	25′	3.64 br s		71.38 (t)
25	3.64 br s		71.43 (t)	26′	4.00 qd	7.0, 1.8	65.92 (t)
26	3.99 qd	7.0, 1.8	65.88 (t)	27′	1.26 d	7.0	16.77 (p)
27	1.24 d	7.0	16.74 (p)	9′-OH	2.63 d	3.7	
9-OH	3.98 br s			24′-OH	2.04 br s		
11-OH	5.22 d	2.0		25′-OH	1.95 br s		
24-OH	2.03 br s						
25-OH	1.95 br s						

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[a] ^1H chemical shift values (δ ppm from $SiMe_4$) followed by multiplicity and then the coupling constants (J/Hz).

[b] Letters, p, s, t and q in parentheses indicate respectively primary, secondary, tertiary and quaternary carbons, assigned by DEPT.

hydroxy groups. The ^1H–^1H COSY analysis of **1** led to five partial structural units (A, B, C×2 and D) as shown by bold-faced lines in Fig. 1. These results were supported by HMBC correlations. The geometrical configuration of both the conjugated diene moieties (C-2–C-5 and C-2′–C-5′) was deduced as *trans-s-trans* from the coupling constants of the olefinic protons and NOEs (H-3/H-5 and H-3′/H-5′). The connection of these five units and the remaining functional groups was determined on the basis of the key HMBC correlations summarised in Fig. 1, and the planar structure of **1** was elucidated.

The relative stereochemistry for **1** was established by a combination of observed coupling constants and

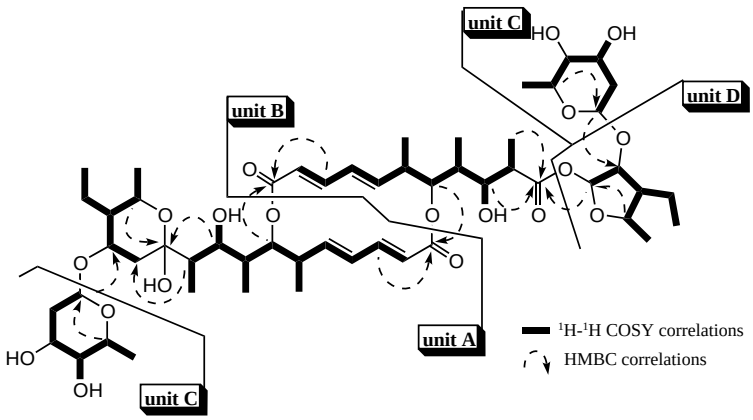


Figure 1. Typical 2D NMR correlations in halichoblelide (**1**).

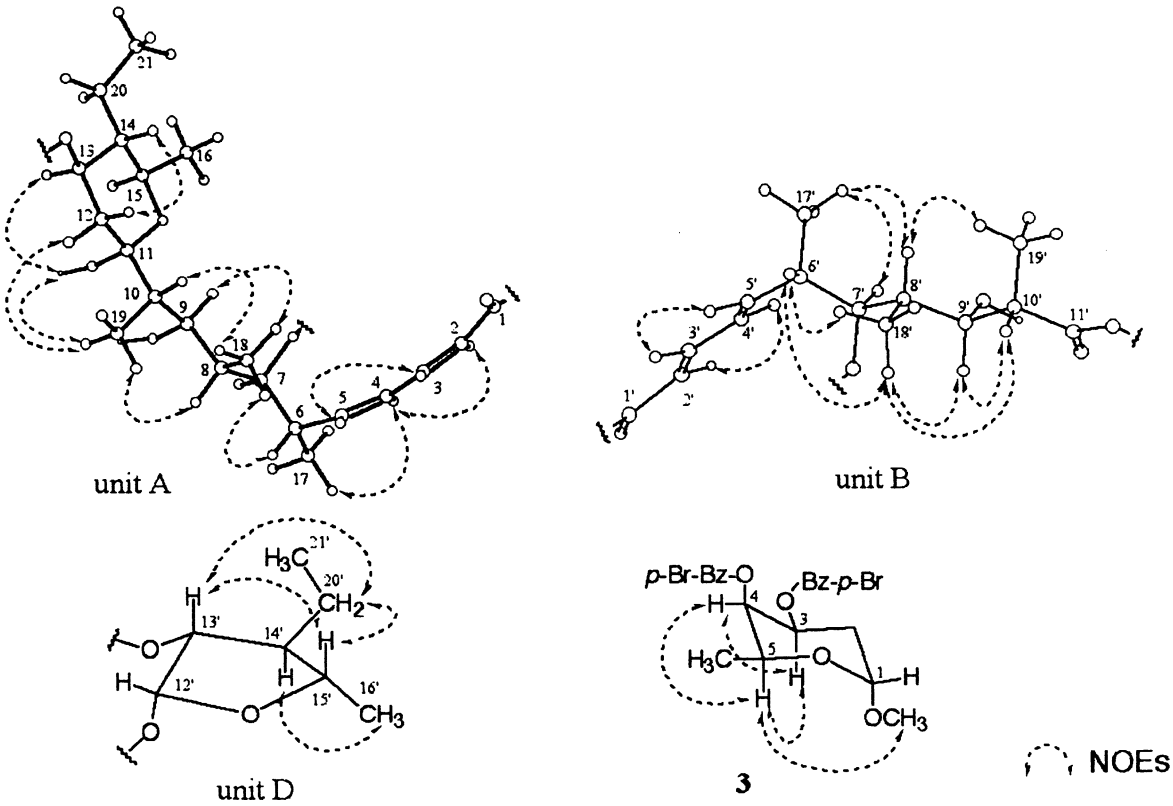
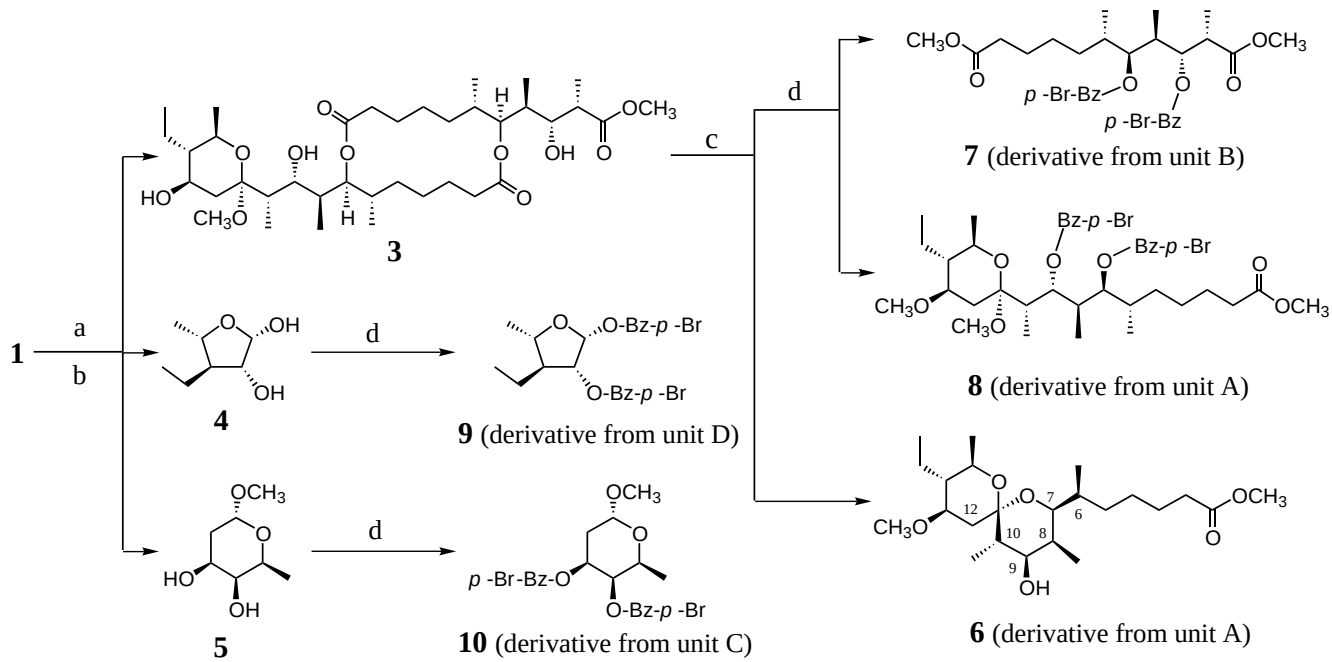


Figure 2. The relative configuration and conformation of the partial structural units and derivative **3**, and observed NOEs.

NOESY data of **1** and the products which were obtained by the degradation of **1** involving several reaction steps shown in Scheme 1. The observation of the coupling constants ($J_{9,10}=0.8$ Hz and $J_{8,9}=10.3$ Hz) and NOEs for H-10/H-18, H-9/H-18 and H-8/H-19 in unit A (Fig. 2) suggested that H-9 and H-10, and H-8 and H-9 are in *gauche* and *anti* arrangements, respectively. Similarly, the $J_{7,8}$ and $J_{6,7}$ values (1.5 and 10.3 Hz, respectively) and NOEs for H-6/H-18 suggested that H-7 and H-8, and H-6 and H-7 are located in *gauche* and *anti* arrangements, respectively. NOEs for H-12β/H-14 and H-13/11-OH implied that the pyranose



Scheme 1. *Reagents and conditions*: (a) Pd–C/H_2, rt; (b) *p*-TsOH/MeOH, rt; (c) conc. H_2SO_4/MeOH, rt; (d) *p*-BrBzCl/pyridine, rt.

ring exists in a chair conformation with H-13 and 11-OH in coaxial arrangements. Furthermore, the $J_{13,14}$ and $J_{14,15}$ values (both 10.2 Hz) suggested that H-13, H-14 and H-15 are oriented *trans*-axially the one to another. This stereochemistry of unit A was supported by stereochemical analysis of degraded product **6** of halichoblelide (**1**) (Scheme 1). The observation of the coupling constant ($J_{6,7}=10.1$ Hz) and NOEs for H-10/H-18, H-9/H-7, H-9/H-12α, H-17/H-8, H-17/H-18 and H-6/H-18 in **6** allowed assignment of the relative configuration of **6** as shown in Scheme 1.

The measured coupling constants and NOEs in unit B (Fig. 2) closely resembled those of unit A. The $J_{9',10'}$ and $J_{8',9'}$ values (3.6 and 10.3 Hz, respectively) and NOEs for H-10′/H-18′, H-9′/H-18′ and H-8′/H-19′ implied that H-9′ and H-10′, and H-8′ and H-9′ are located in *gauche* and *anti* arrangements, respectively. Similarly, the $J_{7',8'}$ and $J_{6',7'}$ values (1.6 and 10.3 Hz, respectively) and NOEs for H-6′/H-18′, H-8′/H-17′ and H-7′/H-17′ suggested that H-7′/H-8′ and H-6′/H-7′ are in *gauche* and *anti* arrangements, respectively.

Unit D and degraded product **9** of halichoblelide (**1**) (Scheme 1) exhibited similar coupling constants and NOEs. The observation of an NOE between H-13′ and H-15′ in unit D implied that H-13′ and H-15′ are in a co-pseudoaxial arrangement. In addition, NOEs from H-20′, but not from H-14′, to H-13′ and H-15′ indicated and H-20′ is arranged *cis* to H-13′ and H-15′. The observation of the coupling constants ($J_{12',13'}=3.0$ Hz in unit D and $J_{1,2}=3.8$ Hz in **9**) indicated that H-12′ and H-13′ in unit D (H-1 and H-2 in **9**) are orientated *cis* on the basis of comparison of coupling constants with α- and β-galactofuranoses.[4] This evidence allowed assignment of the stereochemistry of unit D as shown in Fig. 2.

The stereochemistry of unit C was elucidated by analysis of coupling constants and NOE signals in degraded product **10** of halichoblelide (**1**) (Scheme 1). The same stereochemistry of the anomers in **10** and unit C was deduced from the fact that both the *J* values from the anomeric proton to the two vicinal protons were small (1.8–3.0 Hz) in **10** and unit C. The *J* values ($J_{1,2\beta}=1.8$ Hz, $J_{1,2\alpha}=3.0$ Hz and $J_{3,4}=J_{4,5}=2.2$ Hz) and NOEs from H-5 to H-3 and 1-OCH_3, and from H-4 to H-5 and 5-CH_3 in **10** implied that 3-OH, 4-OH and 5-CH_3 in **10** are orientated *cis* to one another and opposite to 1-OCH_3. This measurements evidently lead to the relative configuration of unit C.

The absolute configuration of halichoblelide (**1**) was elucidated by application of the modified Mosher's method for degraded product **6** of **1** and CD spectra of the other products. The ^{1}H chemical-shift differences between the (*R*)- and (*S*)-MTPA esters of **6** showed that C-9 has an *R*-configuration. Bis-*p*-bromobezoate **8** with the same absolute configuration as **6** exhibited a positive Cotton effect in the CD spectrum. Based on this evidence, a positive Cotton effect observed in the CD spectrum of bis-*p*-bromobezoate **7** allowed assignment of the absolute configuration of **7** as shown in Scheme 1. In addition, compound **10** exhibited a negative cotton effect while compound **9** showed a positive Cotton effect, thus allowing the assignments of their absolute configuration represented in Scheme 1. The absolute configuration of **6**, **7**, **9** and **10** led to determine the structures of four units A, B, D and C, respectively, and consequently the absolute stereostructure for halichoblelide (**1**) was elucidated.

The biological activity tests of halichoblelide (**1**) showed that this new product exhibited potent cytotoxic activity against the murine P388 cell line (ED_{50} 0.63) and the 39 human cancer cell lines (BSY-1, NCI-H522, MKN74 and the other. The tested mean value of log GI_{50} over all cell lines appeared to be −5.25).

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2. Takahashi, C.; Takada, T.; Yamada, T.; Minoura, K.; Uchida, K.; Matsumura, E.; Numata, A. *Tetrahedron Lett.* **1994**, *35*, 5013–5014.
3. Halichoblelide A (**1**): colourless powder, mp 137–139°C, $[\alpha]_D$ −16 (*c* 0.16, EtOH); UV λ_{max} (EtOH)/nm: 252 (log ε 4.56); IR ν_{max} (KBr)/cm^{-1}: 3425, 1722, 1692, 1645, 1638; HRSIMS *m*/*z*: 1029.5035 [M−2CH_3−H+Na]$^+$ (calcd for $C_{52}H_{78}O_{19}Na$: 1029.5036). Methyl acetal derivative **2**: colourless powder, mp 143–145°C, $[\alpha]_D$ −38.6 (*c* 0.12, EtOH); HRSIMS *m*/*z*: 1074.2154 [M+Na]$^+$ (calcd for $C_{55}H_{87}NaO_{19}$: 1074.2156).
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