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Gymnastatins, Novel Cytotoxic Metabolites Produced by a Fungal Strain from a Sponge

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Abstract : Gymnastatins A-C, produced by a strain of *Gymnasella dankaliensis* from the sponge *Halichondria japonica*, are novel compounds with significant cytotoxicity against tumour cells in culture. Their stereostructures have been established on the basis of spectral analyses.
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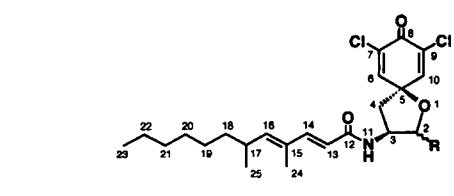
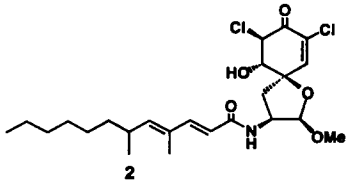
In our continuing search for antitumour and/or cytotoxic metabolites from microorganisms inhabiting the marine environment,[1] we have found that three novel cytotoxic compounds, designated gymnastatins A (**1**), B (**2**) and C (**3**), are produced by a strain of *Gymnasella dankaliensis* which was isolated from the sponge *Halichondria japonica*. The producing microorganism was cultured at 27°C for 4 weeks in a medium (90 l) containing 1% malt extract, 1% glucose and 0.05% peptone in artificial seawater adjusted to pH 7.5. The MeOH extract of the mycelia was purified by bioassay-directed fractionation employing a combination of Sephadex LH-20 and silica gel column chromatographies and reverse phase HPLC to afford gymnastatins A (**1**)(57 mg), B (**2**)(11 mg) and C (**3**)(10 mg) as colorless powder.

Gymnastatin A (**1**)[2] was assigned a molecular formula of $C_{23}H_{31}NO_4Cl_2$ as deduced from HREIMS of **1** and its acetate (**4a**) described below. The presence of two chlorine atoms was supported by the ratio of intensity of isotope peaks ($[M+2]^+$ and $[M+4]^+$). This compound exhibited complex ^1H and ^{13}C NMR spectra,[2] suggesting that it existed in a 2 : 1 mixture of two stereoisomers (**1a** and **1b**) which could not be separated by various column chromatographies. Acetylation of **1** afforded two separable monoacetates (**4a**[3]



1 R=OH
4a R=OAc (*S**)
4b R=OAc (*R**)



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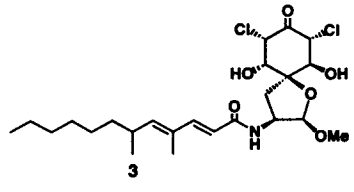

3

and **4b**). The ^{1}H and ^{13}C NMR signals for acetoxy methine groups in **4a** and **4b** appeared at δ_H 6.37 and δ_C 95.2, and δ_H 6.38 and δ_C 101.2, respectively. Additionally, hydrolysis of **4a** by an aqueous ammonia solution gave **1** (a 2 : 1 mixture of two stereoisomers), implying that two stereoisomers are at equilibrium in **1** with a free hydroxy group. From these facts it was deduced that **1** had a hemiacetal group and was a mixture of stereoisomers on its hydroxy group.

A close inspection of the ^{1}H and ^{13}C NMR spectral data (Table 1) of **4a** by DEPT and ^{1}H-^{13}C COSY experiments revealed the presence of three methyls including one primary, secondary and vinyl methyl each, six methylenes, two sp^3-hybridized methines including one methine linked to a nitogen atom, one disubstituted and three trisubstituted double bonds, a secondary amide and a quaternary sp^3-carbon linked to an oxygen atom in addition to the acetoxymethine of the hemiacetal group. The remaining functionality, corresponding to the carbon signal at δ_C 172.2, was shown to be a ketone in a cross-conjugated dienone system on the basis of long-range (LR) ^{1}H-^{13}C COSY correlations (C-8/H-6 and C-8/H-10). The carbon signal of the conjugated ketone appeared shifted upfield by ca. 10 ppm, relative to a general cross-conjugated cyclohexadienone (δ_C 183-185)[4-6], suggesting that chlorine atoms exist at the α-position of the ketone.[6,7] Thus, partial structure A (C-6 to C-10) was established. The ^{1}H-^{1}H COSY analysis of **4a** led to other partial structures B (C-2 to C-4, N-11 and C-12) and C (C-13 to C-25), which were supported by LR ^{1}H-^{13}C COSY correlations. The geometry of the diene in partial structure C was deduced from coupling constants of olefinic protons, a chemical shift of the ^{13}C NMR signal of a vinyl methyl,[8] and NOEs (H24/H13 and H14/H16). The connection of the partial structures (A to C) and the remaining functional group (the quaternary sp^3-carbon linked to an oxygen atom) was determined on the basis of LR ^{1}H-^{13}C COSY correlations (C5/H6, C10/H6, C10/H4 and C12/H14). Based on this evidence, the planar structure of **4a** was elucidated. The NOEs (H3/H2, H3/H4B, H3/H10 and H6/H4A) observed in addition to those mentioned above led to the relative stereostructure **4a** with the 2*S** and 3*S** configurations. Consequently, the stereostructure of **1** as a mixture of diastereomers at the 2 position was established except for the configuration of the 17 position.

Gymnastatin B (**2**)[9] had the molecular formula $C_{24}H_{35}NO_5Cl_2$ as established by HREIMS. A close inspection of its ^{1}H and ^{13}C NMR spectra (Table 1) revealed that the proton and carbon signals (H6, C6 and C7) of one trisubstituted double bond in **1** were missing from **2** and replaced by those of two sp^3-methines bearing a chlorine atom and a hydroxy group [δ_H 4.29 (H6) and δ_C 75.5 (C6), and δ_H 5.38 (H7), and δ_C 60.9 (C7)]. In addition, the carbon signal of the ketone in **2** was found shifted lowfield (δ_C 183.4), relative to **1**, and a signal for a methoxy group newly appeared in the spectrum of **2**. This evidence led to the planar structure of **2** which was supported by HMBC correlations. The relative configuration of the spiro-ring in **2** was established by the NOESY experiment. NOEs from H3 to H2, H4B and H10 showed these protons to be on the same side while NOEs from H6 to H4A and OMe, and from H7 to 6-OH implied that H6 is oriented at the same side as OMe and has a *pseudoequatorial* configuration, and H7 is oriented *trans* to H6. Thus, the relative stereostructure of **2** was established.

Gymnastatin C (**3**)[10] was assigned the molecular formula $C_{24}H_{37}NO_6Cl_2$ as deduced from HREIMS. The general features of the ^{1}H and ^{13}C NMR spectra (Table 1) of **3** closely resembled those of **2** except that the proton and carbon signals of one trisubstituted double bond (C9 and C10) in **2** were replaced by those of two sp^3-methines bearing a chlorine atom and a hydroxy group [δ_H 4.83 (H9) and δ_C 66.7 (C9), and δ_H 4.13 (H10), and δ_C 74.0 (C10)] in **3** and the carbon signal of the ketone in **3** was found shifted lowfield (δ_C 190.5),

Table 1. ^{1}H and ^{13}C NMR data of compounds **2**, **3** and **4a** in $CDCl_3$[a]

	2		**3**		**4a**	
Position	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
2	4.66 d (3.5)	96.6 (t)	4.67 d (3.6)	97.1 (t)	6.37 d (4.4)	95.2 (t)[b]
3	4.09 dddd (12.4,8.7,4.7,3.5)	46.4 (t)	4.21 dddd (12.3,8.3,5.3,3.6)	46.1 (t)	5.06 dddd (12.9,8.2,7.8,4.4)	51.0 (t)
4A	1.98 dd (12.4, 3.5)	38.5 (s)	1.73 t (12.3)	34.6 (s)	2.22 t (12.9)	38.4 (s)
B	2.25 dd (12.4, 4.7)		2.49 dd (12.3, 5.3)		2.63 dd (12.9, 7.8)	
5		69.1 (q)		71.8 (q)		79.9 (q)
6	4.29 t (2.2)	75.5 (t)	4.34 d (3.6)	74.1 (t)	6.89 d (2.7)	145.1 (t)
7	5.38 d (2.2)	60.9 (t)	5.20 d (3.6)	61.2 (t)		131.7 (q)
8		183.4 (q)		190.5 (q)		172.2 (q)
9		133.2 (q)	4.83 d (10.2)	66.7 (t)		131.3 (q)
10	6.96 d (2.2)	142.9 (t)	4.13 dd (10.2, 5.4)	74.0 (t)	7.06 d (2.7)	143.3 (t)
11	5.81 br. d (8.7)		5.89 br. d (8.3)		5.70 br. d (8.2)	
12		166.6 (q)		166.6 (q)		166.3 (q)
13	5.70 d (15.3)	116.7 (t)	5.71 d (15.3)	116.6 (t)	5.73 d (15.1)	116.1 (t)
14	7.13 d (15.3)	147.7 (t)	7.19 d (15.3)	148.0 (t)	7.18 d (15.1)	148.4 (t)
15		130.7 (q)		130.8 (q)		130.7 (q)
16	5.68 d (10.2)	149.0 (t)	5.69 d (9.8)	149.0 (t)	5.73 d (9.7)	149.2 (t)
17	2.50 m	33.3 (t)	2.50 m	33.3 (t)	2.53 m	33.3 (t)
18	1.37 m	37.2 (s)	1.38 m	37.2 (s)	1.37 m	37.2 (s)
19	1.24 m	27.5 (s)[c]	1.24 m	27.5 (s)[c]	1.24 m	27.5 (s)[c]
20	1.24 m	29.4 (s)[c]	1.24 m	29.4 (s)[c]	1.24 m	29.4 (s)[c]
21	1.24 m	31.8 (s)	1.24 m	31.8 (s)	1.24 m	31.8 (s)
22	1.24 m	22.6 (s)	1.24 m	22.6 (s)	1.24 m	22.6 (s)
23	0.87 t (6.7)	14.1 (p)	0.87 t (6.7)	14.1 (p)	0.86 t (6.6)	14.1 (p)
24	1.76 s	20.5 (p)	1.77 s	20.5 (p)	1.77 s	20.5 (p)
25	0.97 d (6.6)	12.5 (p)	0.97 d (6.5)	12.5 (p)	0.97 d (6.6)	12.5 (p)
2-COCH3					2.21 s	21.3 (p)
2-COCH3						169.1 (q)
2-OMe	3.48 s	55.1 (p)	3.46 s	55.3 (p)		
6-OH	4.89 br. s		2.76 br. s			
10-OH			4.32 br. s			

[a] Measured at 300 and 75.4 MHz for ^{1}H and ^{13}C, respectively. [b] Letters, p, s, t and q, in parentheses indicate respectively primary, secondary, tertiary and quarternary carbons, assigned by DEPT. [c] Assignments interchangeable.

relative to **2**. This observation led to the planar structure of **3** which was supported by HMBC correlations. The relative stereochemistry for **3** was established by a combination of coupling constants and NOE data. NOE observed between H7 and H9 implied that the cyclohexanone ring exists in a chair conformation and the two protons are in a *trans diaxial* orientation. Further NOEs from H6 to H7 and OMe implied that H6 and the methoxy group are on the same side and the former has an *equatrial* configuration while NOE between H2 and H3 showed that these protons have a *cis* configuration. The coupling constant ($J_{9,10}$ 10.2 Hz) between H9 and H10 suggested an *axial* orientation for H10. These observations led to the relative stereostructure **3** for gymnastatin C.

Gymnastatins A (**1**), B (**2**) and C (**3**) exhibited potent cytotoxicity (ED_{50} 0.018, 0.108 and 0.106 mg/ml, respectively) in the P388 lymphocytic leukemia test system in cell culture.[11]

References and Notes

1. a) C. Takahashi, A. Numata, Y. Ito, E. Matsumura, H. Araki and K. Kushida, *J. Chem. Soc., Perkin Trans. 1*, 1859 (1994); b) C. Takahashi, T. Takada, T. Yamada, K. Minoura, K. Uchida, E. Matsumura and A. Numata, *Tetrahedron Lett.*, **35**, 5013 (1994); c) A. Numata, C. Takahashi, Y. Ito, K. Minoura, Yamada, C. Matsuda and K. Nomoto, *J.Chem. Soc. Perkin Trans 1*, 239 (1996).
2. Data for **1** : $[\alpha]_D$ - 3.8° (*c* 0.7, $CHCl_3$) ; UV λ max (EtOH) nm (log ε) 266 (4.63); IR υ max

(KBr) cm^{-1} 3377, 3277, 1697, 1653, 1606; HR EIMS *m/z* 437.1507 ($M^+ - H_2O$), Δ - 1.6 mmu; ^1H NMR (300 MHz, $CDCl_3$) δ 0.87 (t, *J* 6.7 Hz, H23), 0.96 (d, *J* 6.6 Hz, H25), 1.23 (br s, H19-22), 1.37 (m, H18), 2.24(t, *J* 12.9 Hz, H4A), 2.50 (m, H17), 2.59 (dd, *J* 12.9, 8.3 Hz, H4B for **1a**), 2.82 (dd, *J* 12.9, 8.3 Hz, H4B for **1b**), 4.65 (m, H3 for **1b**), 4.79 (m, H3 for **1a**), 5.11 (br s, 2-OH), 5.53 (s, H2 for **1b**), 5.54 (d, *J* 4.3 Hz, H2 for **1a**), 5.78 (d, *J* 9.8 Hz, H16), 5.78 (d, *J* 15.1 Hz, H13), 7.03 (d, *J* 2.7 Hz, H6), 7.14 (d, *J* 2.7 Hz, H10), 7.26 (d, *J* 15.1 Hz, H14); ^{13}C NMR (75.4 MHz, $CDCl_3$) δ 12.5 (C25), 14.1 (C23), 20.5 (C24), 22.6 (C22), 27.5 (C19), 29.4 (C20), 31.8 (C21), 33.2 (C17), 37.2 (C18), 38.3 (C4A for **1a**), 40.1 (C4A for **1b**), 52.2 (C3 for **1a**), 57.9 (C3 for **1b**), 79.0(C5 for **1a**), 80.3 (C5 for **1b**), 96.5 (C2 for **1a**), 103.2 (C2 for **1b**), 116.1 (C13), 130.6 (C7), 130.7 (C9), 130.8 (C15), 144.7 (C6), 147.2 (C10), 147.8 (C14), 149.0 (C16), 167.0 (C12 for **1a**), 167.4 (C12 for **1b**), 172.8 (C8).

3. Data for **4a**: $[\alpha]_D$ - 19.7° (*c* 0.9, $CHCl_3$) ; UV λ max (EtOH) nm (log ε) 266 (4.61); IR υ max (KBr) cm^{-1} 3313, 1756, 1702, 1647, 1624; HR EIMS *m/z* 497.1742 (M^+), Δ 0.8 mmu.
4. T. Higa, *Tetrahedron Lett.*, 26, 2335 (1985).
5. A. Rieker and S. Berger, *Org. Magn. Reson.*, 4, 857 (1972).
6. C. J. Pouchert and J. Behnke, *The Aldrich Library of ^{13}C and ^1H FTNMR Spectra*, Edition 1, Vol. 1, p. 696, 713-716. Aldrich Chemical Company, 1993.
7. M. F. Grenier-Loustalot, P. Iratcabal, A. Forchioni and F. Metras, *Org. Magn. Reson.*, 8, 544 (1976).
8. G. Englert, *Helv. Chim. Acta*, 58, 2367 (1975).
9. Data for **2**: $[\alpha]_D$ - 122.1° (*c* 0.3, $CHCl_3$) ; UV λ max (EtOH) nm (log ε) 265 (4.50); IR υ max (KBr) cm^{-1} 3384, 3275, 1725, 1650, 1610; HR EIMS *m/z* 487.1907 (M^+), Δ 1.7 mmu.
10. Data for **3**: $[\alpha]_D$ - 151.2° (*c* 0.1, $CHCl_3$) ; UV λ max (EtOH) nm (log ε) 265 (4.42); IR υ max (KBr) cm^{-1} 3430, 3322, 1755, 1651, 1604; HR EIMS *m/z* 505.1993 (M^+), Δ - 0.3 mmu.
11. A. Numata, P. Yang, C. Takahashi, R. Fujiki, M. Nabae and E. Fujita, *Chem. Pharm. Bull.*, **37**, 648, (1989).

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