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Comparative Study of Toxic and Non-toxic Cyanobacterial Products: A Novel Glycoside, Suomilide, from Non-toxic *Nodularia spumigena* HKVV

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Abstract: A novel glycosidic compound, suomilide (**1**), was isolated together with 1-(*O*-α-glucopyranosyl)-3,25-hexacosanediol, a "heterocyst glycolipid" from the non-toxic *Nodularia spumigena* HKVV. Their structures were determinated by 2D-NMR techniques and MS/MS experiments. © 1997 Elsevier Science Ltd.

In a preceding paper[1], we showed that peptides other than hepatotoxic peptides are produced together with a hepatotoxic peptide, nodularin, by a toxic cyanobacterium, *Nodularia spumigena* AV1, and reported the isolation and the structural determination of the cyclic and linear peptides, nodulapeptins and spumigins A~C[1,2]. These structures are similar to those of anabaenopeptins[3-5] and aeruginosins[6-8] produced by toxic cyanobacteria, such as *Anabaena*, *Microcystis* and *Oscillatria*. No such peptides were detected in the extract of a non-toxic cyanobacterium, *N. spumigena* HKVV[9], whereas two glycosidic compounds were produced by this strain. We now report the isolation and the structural determination of a novel glycosidic compound, suomilide (**1**), and 1-(*O*-α-glucopyranosyl)-3,25-hexacosanediol, a "heterocyst glycolipid" from this non-toxic *N. spumigena*.

The compound **1** was purified by repeated silica gel and TOYOPEARL HW-40F chromatographies[10] as a colorless amorphous powder: $[\alpha]_D^{26}$ +74.2° (*c* 0.100, MeOH); negative FABMS (matrix: glycerol) *m/z* 1046 $[M-H]^-$. The desulfated ion at *m/z* 968 $[M+H-SO_3]^+$ was observed in the positive FAB mass spectrum, suggesting the presence of a sulfate group. The molecular formula of **1** was established to be $C_{45}H_{73}N_7O_{19}S$ based on the HRFABMS (*m/z* 968.5137 $[M+H-SO_3]^+$ Δ -5.5 mmu) and NMR spectral data (Table 1). It was suggested that **1** is a glycosidic compound containing an amino acid moiety based on the ^1H and ^{13}C NMR spectra and has a D-*allo*-Ile and an amino compound with a molecular weight of 154 by amino acid analysis using the advanced Marfey's method[11]. The 2D-NMR analyses easily indicated the presence of the following segments: *allo*-Ile, α-glucose[12], two hexanoic acids (HA) and 2-*O*-methylglyceric acid 3-*O*-sulfate (Mgs) which was contained in oscillapeptin[13]. The ^{13}C signal (δ 154.1) that correlated to the ^1H signals (4H, 7.13

Table 1. ^{1}H and ^{13}C NMR spectral data for suomilide (**1**) in DMSO-d_6

Position		^{1}H	^{13}C	$\Delta^{13}C$*	Position		^{1}H	^{13}C	$\Delta^{13}C$*
allo -Ile	1		172.0	0.036	Mgs	1		169.9	0.099
	2	4.57	53.3	0.124		2	3.94	80.0	0.037
	3	1.70	36.3	0.019		3	3.75	66.2	0.011
	4	1.17	25.4	0.004			3.94		
		1.29				OMe	3.29 (3H)	57.3	0.025
	5	0.89 (3H)	11.7	0.015	Aacp	2	4.09	55.4	0.025
	6	0.87 (3H)	14.5	0.015			4.11		
	NH	8.03				3		135.9	0.058
α-glucose	1	4.78	94.7	0.007		4	5.61	118.8	0.018
	2	4.35	72.9	0.048		5	4.11 (2H)	54.2	0.018
	3	3.56	69.9	0.065		6	2.23 (2H)	27.7	0.029
	4	3.14	70.2	0.157		7	3.16 (2H)	37.2	0.134
	5	3.66	70.0	0.014		7-NH	7.53		
	6	4.02	63.3	0.019		guanigine	7.13 (4H)	154.1	0.208
		4.29			Azabicyclo moiety	1	4.23	53.5	0.043
	3-OH	5.23				3	4.59	56.6	0.029
	4-OH	5.31				4	4.27	58.1	0.127
HA-1	1		172.8	0.019		5		80.4	0.044
	2	2.30 (2H)	33.2	0.003		6	1.94	34.5	0.029
	3	1.50 (2H)	24.1	0.011			2.27		
	4	1.25 (2H)	30.6	0.014		7	3.62	69.8	0.066
	5	1.25 (2H)	21.8	0.007		8	1.68	28.7	0.007
	6	0.84 (3H)	13.7	0.011			2.14		
HA-2	1		172.9	0.021		9	3.69	65.7	0.117
	2	2.30 (2H)	33.4	0.011		3-CO		168.7	0.083
	3	1.50 (2H)	24.1	0.011		4-NH	7.95		
	4	1.25 (2H)	30.6	0.011		9-OH	6.02		
	5	1.25 (2H)	21.8	0.010		urethane		156.6	0.044
	6	0.84 (3H)	13.8	0.007					

* $\Delta^{13}C$ values are the difference in the ^{13}C chemical shifts between DMSO-d_6 and DMSO-d_6+D_2O.

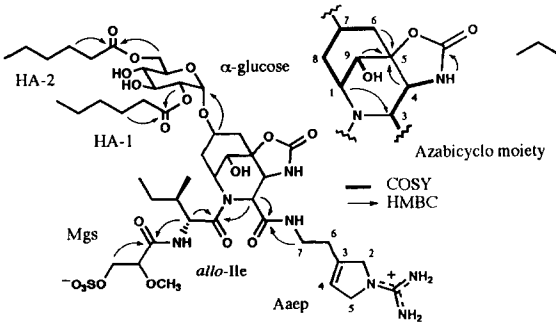

Fig. 1. Structure of suomilide (**1**).

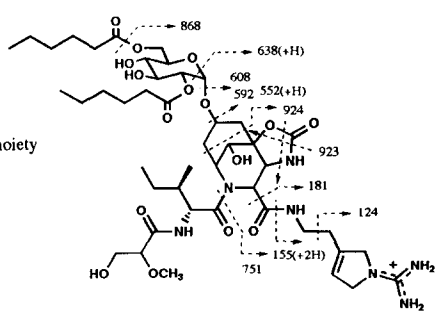

Fig. 2. Product ions for $[M+H-SO_3]^+$ at *m/z* 968.

ppm) in the HMBC spectra showed the existence of a guanidine group. Although the HMBC correlations between the H-2 and -5 of 1-amidino-3-(2-aminoethyl)-3-pyrroline (Aaep) and the guanidine carbon were not observed, the connectivity was deduced on the basis of MS/MS experiments[14] as will be described later. Finally, the structure of Aaep containing a 5-membered ring system was assured on the basis of the chemical shifts of C-2 and C-5 and isotope shift experiments[15] (Table 1).

The structure of the last segment, the azabicyclo moiety, was determined as follows: in the COSY spectrum, two connectivities (solid lines in Fig. 1), from H-3, which was correlated to the carbonyl carbon (δ 168.7) in the HMBC spectrum, to 4-NH and from H-6 to 9-OH, were determined. The HMBC correlations indicated that C-6, C-9 and C-4 of these connectivities were linked to each other *via* the quaternary carbon, C-5 (δ 80.4), as shown in Fig. 1. On the basis of the chemical shifts, C-1 (δ 53.5) and C-3 (δ 56.6) were adjacent to a nitrogen atom, and H-1 was correlated to C-3 in the HMBC spectrum. Therefore, the skeletal structure of this segment was determined to be 4-amino-5,7,9-trihydroxy-2-azabicyclo[3.3.1]nonane-3-carboxylic acid. In addition, the presence of a urethane linkage, in which 4-NH was combined with 5-OH *via* the carbonyl carbon (δ 156.6), was deduced in the azabicyclo moiety based on the HMBC spectrum and the molecular formula of **1**.

The sequence of these segments was established with the help of the HMBC experiments (correlation: Mgs CO/*allo*-Ile H-2, *allo*-Ile CO/azabicyclo moiety H-3, azabicyclo moiety 3-CO/Aaep H-7, α-glucose C-1/azabicyclo moiety H-7, HA-1 CO/α-glucose H-2, HA-2 CO/α-glucose H-6). Thus, these results led to the gross structure of **1** as shown in Fig. 1. The established structure of **1** was further supported by FAB MS/MS experiments[14]. Product ions charge-retained Mgs containing a sulfate group were observed in the negative MS/MS spectrum of $[M-H]^-$ of **1**. On the other hand, product ions charge-retained Aaep containing guanidine were observed in the positive MS/MS spectrum of $[M+H-SO_3]^+$ of **1** as shown in Fig. 2. These results rationally established and definitely reinforced the structure of **1**. Elucidation of the remaining stereochemistries is now in progress.

Furthermore, we also isolated another glycosidic compound, which was identified to be 1-(*O*-α-glucopyranosyl)-3,25-hexacosanediol[16], from the EtOH extract of this cyanobacterium after the 5% AcOH aq. extraction[17]. Recently, Soriente *et al.* reported the isolation and the structural determination of this compound as the "heterocyst glycolipid" from *Nodularia harveyana*[18].

In a preceding study, two types of peptides were isolated together with nodularin from the toxic *N. spumigena*. However, peptides other than hepatotoxic peptides obtained from toxic cyanobacteria were not isolated from the non-toxic *N. spumigena*, whereas a novel glycosidic compound, suomilide (**1**)[19], was isolated together with the glycolipid in the present study. Recently, Namikoshi *et al.* summarized the bioactive compounds produced by cyanobacteria and reported that cyanobacteria produce four other types of peptides in additon to the hepatotoxic peptides: 19-membered cyclic peptides containing an ureido linkage, 19-membered depsipeptides containing the 3-amino-6-hydroxy-2-piperidone (Ahp) unit, linear peptides and depsipeptides of a tricyclic ring system[20]. Although these peptides are produced by almost all toxic cyanobacteria that co-produce hepatotoxic peptides, no such peptides were detected from the neurotoxic cyanobacteria[21], *Anabana* sp. 37, 123 and *Oscillatria* sp. 193, that produce anatoxin-a. On the basis of these

accumulated data, it is further suggested that the production of such peptides is characteristic for the toxic cyanobacteria and is closely related to that of the hepatotoxic peptides.

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17. A fraction (400 mg) containing the glycolipid was obtained from the EtOH extract of residue after the 5% AcOH aq. extraction of dried *N. spumigena* HKVV (5 g) and was separated to give the glycolipid (23.9 mg) by silica gel chromatography using $CHCl_3$:MeOH:H_2O =65:10:5 (lower phase) and $CHCl_3$:MeOH:H_2O =65:20:5 (lower phase).
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