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Stereocalpin A, a bioactive cyclic depsipeptide from the Antarctic lichen *Stereocaulon alpinum*

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Abstract—A new cyclic depsipeptide stereocalpin A (**1**) has been isolated from the MeOH extract of the Antarctic lichen *Stereocaulon alpinum* by various chromatographic methods. The structure of stereocalpin A (**1**) was mainly determined by analysis of the NMR spectroscopic data and by chemical methods. Stereocalpin A (**1**) is a unique cyclic peptide incorporating an unprecedented 5-hydroxy-2,4-dimethyl-3-oxo-octanoic acid in the structure. Stereocalpin A (**1**) shows moderate cytotoxicity against three human solid tumor cell lines.
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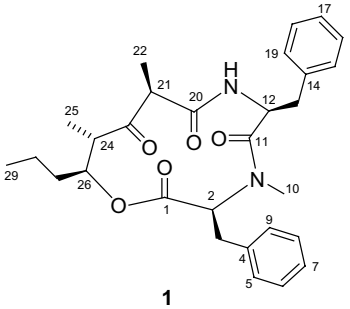
Lichens are symbiotic associations of fungi and algae and/or cyanobacteria that produce unique characteristic secondary metabolites by comparison to those of higher plants.[1–3] Unique lichenous metabolites belong to the chemical classes of depsides, depsidones, and dibenzfuranes, and the production of such compounds by lichens is speculated to be related to their slow growth and harsh living conditions.[2] In addition, a variety of biological activities of lichenous metabolites, including antibiotic, antimycobacterial, antiviral, analgesic, and antipyretic properties, have been revealed in biological screening processes.[1,2] Thus, there is considerable interest in lichenous metabolites as potential sources of pharmacological agents.[4]

In the course of chemical studies of lichens from the Antarctic region as potential sources of new bioactive secondary metabolites, we investigated the MeOH extract of a sample of the lichen *Stereocaulon alpinum*. Chemical studies of this extract led to the isolation of a new cyclic depsipeptide named stereocalpin A (**1**). This report describes the isolation, structure elucidation, and biological activities of compound **1**.



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S. alpinum was collected and identified from Barton Peninsular around King Sejong Station (S 62°13.3′, W 58°47.0′) on King George Island, Antarctica, in January, 2003 by J. H. Yim. Voucher specimens (reference L-5) have been deposited in the Korea Polar Research Institute. The dried sample of *S. alpinum* (50 g) was extracted with MeOH (1 L × 2) for 24 h. The resulting crude MeOH extract (5.9 g) was subjected to C_{18} functionalized silica gel flash column chromatography with a stepwise gradient of 20–100% MeOH in H_2O, and the fraction that was eluted with 90% MeOH was further subjected to purification by semi-preparative reversed-phase HPLC (CH_3CN/H_2O) to yield stereocalpin A (**1**, 13.5 mg).

Keywords: *Stereocaulon alpinum*; Stereocalpin A; Cyclic depsipeptide; Cytotoxicity.
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Stereocalpin A (**1**)[5] was isolated as white powder and analyzed for the molecular formula $C_{29}H_{36}N_2O_5$ by HRESIMS data [*m*/*z* 515.2513 $(M+Na)^+$; Δ −0.9 mmu], and this formula was fully supported by ^1H and ^{13}C NMR data (Table 1). The ^1H NMR and DEPT spectra revealed the presence of four methyl groups, 10 aromatic methines, five sp^3 methines (one oxygenated), and four methylene units. In addition to the signals corresponding to the above carbons, analysis of the ^{13}C NMR spectrum revealed the presence of four carbonyl (one ester group) and two non-protonated aromatic carbons. These structural features accounted for 12 out of 13 unsaturations, and the remaining one must be accounted for by the presence of one additional ring system. Furthermore, the presence of characteristic signals for two α-amino carbons ($\delta_{C/H}$ 50.9/4.97, 58.9/4.66), one *N*-methyl group ($\delta_{C/H}$ 30.0/3.05), and three carbonyl/amide carbonyl carbons (δ_C 167.0, 169.9, 170.6) in the spectra of **1** was indicative of a peptide. Four isolated spin systems corresponding to the C12–C13, C2–C3, C21–C22, and C24–C29/C25 subunits of structure **1** were disclosed by the analysis of ^1H–^1H COSY data, and supported by the analysis of HMQC and HMBC spectroscopic data. Interpretation of HMBC data readily led to the construction of the phenylalanine (Phe; correlations of H-13 with C-11 and of *N*H-12 with C-12), *N*-methyl-phenylalanine (*N*-Me–Phe; correlations of H-2 with C-1, of H-3 with C-1, and of H_3-10 with C-2), and the 5-hydroxy-2,4-dimethyl-3-oxo-octanoic acid (HDMOO) residues (Table 1). The amino acid constituents of **1**, and HDMOO unit, were then connected on the basis of HMBC heteronuclear couplings. The connection between Phe and *N*-Me–Phe residues was demonstrated by correlations from the *N*-methyl protons (H_3-10) to both C-2 and C-11. The amide proton of Phe (δ 5.80) was correlated with the carbonyl carbon (δ_C 167.0) of the HDMOO unit and the α amino carbon of Phe (δ_C 50.9) in the HMBC spectrum. Therefore, the linkage of Phe to the HDMOO was established. Finally, an HMBC correlation of the oxymethine proton (H-26) of HDMOO unit (δ_H 5.30) with the carbonyl carbon of the *N*-Me–Phe residue (δ_C 170.6) led to establishment of the ester linkage between *N*-Me–Phe and HDMOO residues, completing the gross structure of **1** as shown.

The absolute configuration of the chiral centers in **1** was determined by Marfey's method[6] and analysis of NOESY data. The absolute configuration of the amino acid units was partly established by acid hydrolysis (6 N HCl, 120 °C, 24 h) of **1**, followed by derivatization with Marfey's reagent (1-fluoro-2,4-dinitrophenyl-5-L-alanine amide)[6] and subsequent HPLC analysis. By comparing the retention times of authentic standards of L- and D- forms of Phe and *N*-Me–Phe, the hydrolyzate was identified to contain L-Phe and L-*N*-Me–Phe. Further chemical study to assign the absolute configuration at the HDMOO unit was not pursued due to the limited quantity of material available. Instead, the absolute configuration of the HDMOO unit was proposed by

Table 1. NMR spectroscopic data for Stereocalpin A (**1**) in $CDCl_3$

Position	δ_H (int., mult., *J* in Hz)[a]	δ_C[b]	Key NOESY	HMBC (H → C#)
1	—	170.6		
2	4.66 (1H, dd, 9.9, 5.9)	58.9	H-12	C-1, C-3, C-4, C-10, C-11
3	2.99 (1H, dd, 14.2, 4.0)	36.0		C-1, C-2, C-4, C-5/9
	3.11 (1H, m)			
4	—	135.8		
5/9	7.18–7.32 (m)	128.9		
6/8	7.18–7.32 (m)	128.2		
7	7.16–7.23 (m)	126.6		
10	3.05 (3H, s)	30.0		C-2, C-11
11	—	169.9		
12	4.97 (1H, m)	50.9	H-2	
13	2.80 (1H, dd, 14.3, 8.8)	38.4	12-*N*H	C-11, C-12, C-14, C-15/19
	3.14 (1H, m)			
14	—	137.0		
15/19	7.06–7.14 (m)	129.6		
16/18	7.18–7.32 (m)	128.7		
17	7.06–7.14 (m)	127.3		
20	—	167.0		
21	3.87 (1H, q, 7.0)	52.4	H_3-25	C-20, C-22, C-23
22	0.99 (3H, d, 7.0)	15.0	12-*N*H	C-20, C-21, C-23
23	—	210.7		
24	2.57 (1H, m)	48.8		C-23, C-25, C-26
25	1.30 (3H, d, 7.3)	10.4	H-21, H-26	C-23, C-24, C-26
26	5.30 (1H, m)	76.3	H_3-25	C-1, C-23, C-25, C-27
27	1.27 (1H, m)	32.8	H-24	C-24, C-26, C-28, C-29
	1.48 (1H, m)			
28	1.18 (2H, m)	18.9		C-26, C-27, C-29
29	0.89 (3H, t, 7.3)	13.9		C-27, C-28
12-*N*H	5.80 (1H, d, 9.9)	—	H-13, H_3-22	C-12, C-20

[a] Recorded at 400 MHz.
[b] Recorded at 100 MHz.

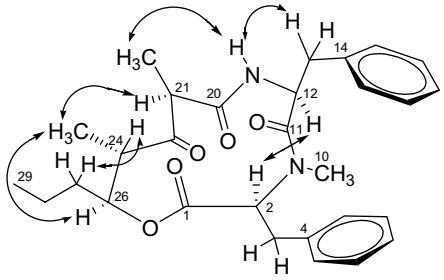

Figure 1. Key NOESY correlations (↔) observed in **1**.

the analysis of NOESY data (Table 1 and Fig. 1) using the L-Phe and L-*N*-Me–Phe as an anchor point. Specifically, the significant NOE observed between two α-protons of Phe and *N*-Me–Phe was consistent with the assignments of L-configurations of the respective amino acid residues. The *N*H (δ_H 5.80) proton of L-Phe residue showed NOESY correlations with one (δ_H 2.80) of the β-protons of L-Phe and with H_3-22 (δ_H 0.99) of HDMOO unit. These data suggested that these protons are on the same side of the macrocyclic ring (β-face of the ring). NOESY correlations of H-21 (δ_H 3.87) with H_3-25 (δ_H 1.30) and of H_3-25 with H-26 (δ_H 5.30) suggested that the respective groups are on the same side of the macrocyclic ring (α-face of the ring), leading to the assignment of the remaining configurations at C-24 and C-26. Taken together, the overall absolute configuration of stereocalpin A (**1**) could be assigned as 2*S*, 12*S*, 21*R*, 24*S*, and 26*S*, though this is not considered to be definitive.

Stereocalpin A (**1**) appears to be derived from two distinctive biosynthetic pathways. The peptide portion of the molecule is clearly derived from non-ribosomal peptide synthetase pathways (NRPS), while the 5-hydroxy-2,4-dimethyl-3-oxo-octanoic acid (HDMOO) unit has a polyketide synthase (PKS) origin.[7] To our best knowledge, the HDMOO unit of stereocalpin A (**1**) has not been previously reported as a component presented in any secondary metabolites.

Stereocalpin A (**1**) was evaluated for cytotoxicity against three human solid tumor cell lines, and marginal levels of cytotoxicity were observed against human colon carcinoma cell line (HT-29, $IC_{50} = 6.5$ μM), human skin carcinoma cell line (B16/F10, $IC_{50} = 11.9$ μM), and human liver carcinoma cell line (HepG2, $IC_{50} = 13.4$ μM). In addition, stereocalpin A (**1**) weakly inhibited the activity of protein tyrosine phosphatase 1B (PTP1B)[8–13] in a dose-dependent manner with IC_{50} value of 40.0 μM.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2007.11.022.

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