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NOTE

A new pseudodepsidone from the Antarctic lichen *Stereocaulon alpinum* and its antioxidant, antibacterial activity

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Several species of lichens have been used for various remedies in folk medicine since ancient time and variety of biologically active lichen metabolites, including antibiotic, antimycobacterial, antiviral, analgesic, antioxidant and antipyretic properties, have been described.[1] During surviving strategy against extreme environment, especially, high UV, Antarctic plants have been believed to produce unique types of metabolites.[2] Several lichen species of the Antarctic origin have shown antibacterial and antioxidant activities.[3–5] In our previous studies of isolation of bioactive metabolites from Antarctic lichens, lobaric acid and related metabolites showing protein tyrosine phosphatase 1B inhibitory activities have been isolated from *Stereocaulon alpinum* of Antarctic origin.[6] Moreover, antibacterial and antioxidant activity showing compounds: ramalin and three new derivatives of usnic acids were isolated from *Ramalina trebrata*.[7,8] In the course of our continuing study in this area, a structurally new pseudodepsidone-type metabolite, lobastin, was encountered from the new collection batch of *S. alpinum* from Antarctica. This report describes the isolation, structure elucidation and comparative biological activities including antimicrobial and antioxidant activities of the lobastin encountered in this study.

Antimicrobial activities were evaluated by determining zone of inhibition and MIC against *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis*, *Candida albicans* and *Aspergillus niger*. Antioxidant activity was estimated by determining DPPH (1-diphenyl-2-picryl-hydazil) free radical-scavenging capacity.[8,9] Brine shrimp lethality test[10] was performed to evaluate the toxicity of the compounds (detail in Supplementary Information).

The compound **1** was isolated as white powder and identified as lobaric acid by analysis of MS and NMR data.[11] Compound **2** (Figure 1, Supplementary Figures S1–8) was isolated as white powder. The compound was optically inactive. The molecular formula was established as $C_{25}H_{28}O_8$ on the basis of HR-ESIMS data for m/z 455.1701 $[M\text{-}H]^-$ with 12 degrees of unsaturation. IR spectrum showed the presence of hydroxyl (3232 cm^{-1}) and carbonyl (1740 cm^{-1}) functionalities. The ^1H NMR spectrum clearly showed the presence of four aromatic/olefinic protons, a number of aliphatic protons and a methoxy group. The ^1H NMR data could not fully show the total proton number as shown in molecular formula, because some replaceable protons were not observed and some protons related to aliphatic chains were overlapped. The ^{13}C NMR data clearly showed the presence of 25 carbon signals including 14 olefinic carbons, suggesting the presence of a minimum of two aromatic rings. In addition, two carbonyl, one methoxy, two methyl and six methylene carbons were clearly observed. The structure of **2** was mainly elucidated based on the analysis of 2D NMR. The partial structures C-9 to C-12 and C-8′ to C-12′ were deduced from the careful analysis of the ^1H–^1H COSY and HSQC data of **2**, along with comparisons of respective data with those for lobaric acid. The HMBC correlation of H-3 to C-1, C-2, C-3, C-5, C-7; H-5 to C-1, C-2, C-3, C-4, C-8 and H-3′ to C-1′, C-2′, C-4′, C-5′, C-7′ indicated the presence of two aromatic rings. The HMBC correlation of olefinic proton H-9 to C-6 and C-8 together with HMBC correlation of methylene proton H-10 to C-8, C-9, C-11, C-12 (Table 1) confirmed the connection of a side chain with the olefinic carbon C-9. In fact, the structure of compound **2** was very close to that of previously encountered pseudodepsidone-type compound **3**[6] (Figure 1) and sakisacaulon A.[12] Therefore, the presence of benzofurane moiety and biphenyl ether connection between C-2 and C-5′ were supported by comparision of the NMR data to those for compound **3** and sakisacaulon A. The absence of oxygenated methyl group at C-8 and the presence of a double bond in between C-8 and C-9 in compound **2** were the structural differences with compound **3**. The observed NMR data clearly supported these structural differences in compound **2** with compound **3** and sakisacaulon A. Finally, NOESY correlation of H-5 with H-9 indicated that the double bond at C-8 and C-9 was in Z configuration, completing the gross structure of **2** as

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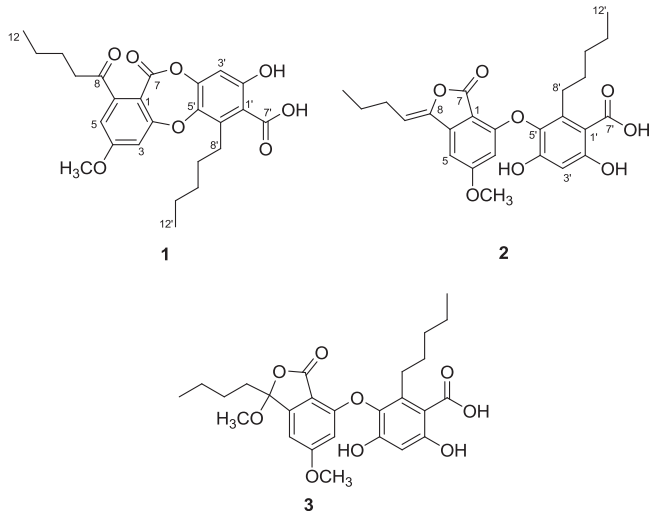

Figure 1 Structures of compounds **1**–**3**.

Table 1 NMR data for compound 2 (400 MHz, DMSO-*d6*)

Position	δ_C	δ_H, *mult. (J in Hz)*	*HMBC*[a]
1	104.2	—	—
2	157.4	—	—
3	101.7	5.90, d (1.8)	1, 2, 4, 5, 7
4	166.4	—	—
5	96.7	7.19, d (1.8)	1, 2, 3, 4, 8
6	143.4	—	—
7	163.2	—	—
8	144.9	—	—
9	109.3	6.00, t (7.7)	6, 8, 10, 11
10	27.3	2.36, m	8, 9, 11, 12
11	21.9	1.52, m	9, 10, 12
12	13.6	0.96, t (7.0)	10, 11
1′	108.4	—	—
2′	158.2	—	—
3′	101.9	6.41, s	1′, 2′, 4′, 5′, 7′
4′	153.2	—	—
5′	131.8	—	—
6′	137.3	—	—
7′	171.2	—	—
8′	27.5	2.70, m 2.52, m	—
9′	29.5	1.39, m 1.27, m	—
10′	31.4	1.12, m	—
11′	21.4	1.12, m	—
12′	13.56	0.69, t (7.0)	10′, 11′
4 –OCH_3	56.3	3.79, s	4
4′-OH	—	10.39, s	3′, 4′, 5′

Abbreviation: DMSO-*d6*, Dimethyl sulfoxide-*d6*.
[a]HMBC correlations, optimized for 8 Hz, are from proton(s) stated to the indicated carbon(s).

shown. The structural similarity of compound **2** with lobaric acid **1** and compound **3** led to the speculation that compound **2** might be an artifact produced from either **1** or **3** during isolation process from the extract of *S. alpinum*. Therefore, direct HPLC-MS analysis of freshly prepared crude extract of *S. alpinum* was conducted in 6310 Agilent Ion Trap LC/MS, and the analysis (Supplementary Figure S9) indicated that the compound **2** (peak at 17.1 min) was present in the crude extract as 16.3% (w/w). Similarly, compounds **1** (peak at 22.5 min) and **3** (peak at 17.7 min) were contained as 25.4 and 7.4%, respectively. As the freshly prepared extract contained significant amount of compound **2**, we concluded that compound **2** was not an artifact but a new natural product.

Table 2 Antibacterial, antioxidant and toxicity activity of compounds 1 and 2

Compound 1	*Compound 2*	*Ampicillin*	*BHA*	*Berberine chloride*	*Remarks*
Inhibition zone (mm 100 μg^{-1} per disk)					
14	20				Against *B. subtilis*
13	16				Against *S. aureus*
MIC (μM)					
88	44	2.7			Against *B. subtilis*
39.6	35.2	2.7			Against *S. aureus*
DPPH activity (μM)					
No activity	70.2		26.6		
(BST) (LC_{50}) (μM)					
No activity	No activity			22.6	

Abbreviations: BHA, butylated hydroxyanisole; BST, Brine shrimp lethal test; Compound **1**, lobaric acid; Compound **2**, lobastin; ; DPPH, 1-diphenyl-2-picryl-hydazil.

In the present experiment, lobaric acid **1** and lobastin **2** were active against Gram-positive bacteria, *B. subtilis* and *S. aureus* (Supplementary Figures S10–12). The paper disk diffusion assay (100 μg/disk) showed that the average zone of inhibition for compounds **1** and **2** were 14 and 20 mm against *B. subtilis*, and 13 and 16 mm in diameter against *S. aureus*, respectively. In addition, the MIC of compounds **1** and **2** were obtained as 88 and 44 μM against *B. subtilis*, and 39.6 and 35.2 μM against *S. aureus*, respectively. Both compounds **1** and **2** did not show antimicrobial activity against Gram-negative bacteria, *C. albicans* and *A. niger*.

Compound **2** reduced DPPH free radicals in dose-dependent manner. The obtained data (Table 2) indicated that compound **2** showed moderate antioxidant activity compared with the synthetic commercial standard butylated hydroxyanisole. Compound **1** did not show DPPH-reducing activity even at higher dose (4000 μM). Perhaps, the presence of double bond in between C-8 and C-9, and opening of heterocyclic ring in compound **2** were responsible for such antioxidant activity of the molecule.

Both compounds **1** and **2** were tested with Brine shrimp larvae at various concentrations (0–4000 μM). Both compounds did not show toxicity effects (Table 2). The commercial standard berberine chloride showed LC_{50} at 22.6 μM. In conclusion, this new compound **2**, lobastin, showed moderate antioxidant and antibacterial activities. It did not show toxic effect to *Artimia salina* larvae. These data may suggest the possible use of this natural compound, lobastin, in future antioxidant therapy.

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