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Antimitotic activity of lobaric acid and a new benzofuran, sakisacaulon A from *Stereocaulon sasakii*

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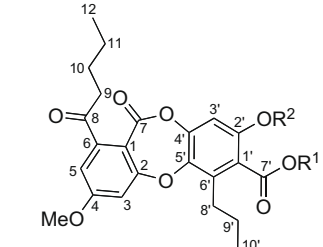
ABSTRACT

Lobaric acid (**1**) has been isolated from lichen, *Stereocaulon sasakii* together with a new benzofuran, sakisacaulon A (**2**). Lobaric acid (**1**) inhibited the polymerization of tubulin. Structure–activity relationship of lobaric acid and its derivatives on inhibitory activity of tubulin polymerization was discussed.

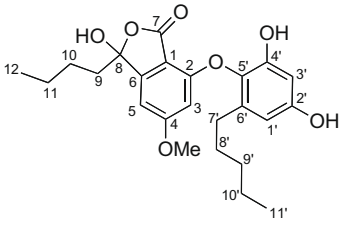
Tubulin and microtubule proteins formed by the self-association of the α,β-tubulin heterodimers interact with a large number of structurally diverse natural products from natural sources, which cause cells to arrest in mitosis.[1] These antimitotic agents are of interest for the insight they can provide potential activity in the treatment of cancer diseases.[2] Among them, paclitaxel is potent inhibitor of cell proliferation and arrest cells in mitosis, but in contrast to vinblastine, promote the polymerization of tubulin, causing stabilization and bundling of microtubules.[3] Recently much effort has been directed to the isolation and synthesis of new antimitotic drugs that target the tubulin/microtubule system and display efficacy against drug-refractory carcinomas.[4] The antimitotic agents have potential applications in drug development.

In our search for bioactive compounds targeting the tubulin/microtubules from natural sources,[5] we found that the extract from lichen, *Stereocaulon sasakii* remarkably inhibited the polymerization of tubulin. Lichens are symbiotic associations of fungi and algae and are known to contain unique substances such as depsides and depsidones.[6] Our efforts on identifying new agents that target tubulin resulted in the isolation of lobaric acid (**1**)[7] from the whole bodies of *S. sasakii* together with a new benzofuran, sakisacaulon A (**2**), whose structures were established by spectroscopic data. This Letter describes effects of a lobaric acid and its derivatives (**4**–**7**) on tubulin assembly as well as structure elucidation of sakisacaulon A (**2**).

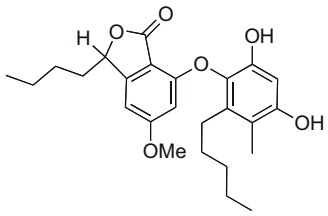
The whole bodies of *S. sasakii* were extracted with MeOH, and the MeOH extract was in turn partitioned with hexane, EtOAc,



lobaric acid **1**: R1 = H, R2 = H
4: R1 = Me, R2 = H
5: R1 = H, R2 = Ac
6: R1 = Me, R2 = Ac
7: R1 = H, R2 = COEt



sakisacaulon A (**2**)



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and *n*-BuOH. The EtOAc-soluble materials inhibiting the polymerization of tubulin were subjected to a silica gel column (CH_2Cl_2/MeOH, 1:0→0:1) followed by a C_{18} column (CH_3OH/H_2O, 3:2→1:0) and C_{18} HPLC (CH_3OH/0.1% TFA, 4:1) to afford lobaric acid (**1**, 0.2%) and a new benzofuran, sakisacaulon A (**2**, 0.004%).

The structure of **1** was identical with lobaric acid by 2D NMR analysis.[7] Lobaric acid (**1**) was crystallized from methanol-water as colorless needles, mp 196–198 °C and was analyzed by X-ray crystallography.[8] The asymmetric unit contains one molecule of **1**, giving a calculated density of 1.387 g cm^{-3}. The ORTEP drawing of **1** was shown in Figure 1. The conformation of the two aromatic rings (C-1–C-6 and C-1′–C-6′) is essentially twisted at 125.28° with extended antiparallel side chains at C-6 and C-6′. This conformation in **1** obtained from X-ray analysis corresponded well to those in the case of the other depsidones such as excelsione and garcidepsidone A.[9]

Compound **2** showed the pseudomolecular ion peak at *m/z* 453 $(M+Na)^+$ in the ESIMS, and the molecular formula $C_{24}H_{30}O_7$ was established by HRESIMS [*m/z* 453.1890 $(M+Na)^+$].[10] IR absorptions implied the presence of hydroxyl (3360 cm^{-1}) and carbonyl (1740 cm^{-1}) functionalities. The ^1H NMR data (Table 1) showed the presence of four aromatic protons, a pentyl and a butyl side chains, and a methoxy group. The ^{13}C NMR data (Table 1) revealed twenty-four carbon signals due to eight sp^2 quaternary carbons, four sp^2 methines, one ester carbonyl, one sp^3 quaternary carbon, seven sp^3 methylenes, one methoxy, and two methyl groups.

Partial structures C-9 to C-12 (unit **a**) and C-7′ to C-11′ (unit **b**) were deduced from detailed analysis of the ^1H–^1H COSY spectrum of **2** (Fig. 2). The HMBC cross-peaks of H-5 to C-1 and C-4, H-3 to C-1, C-2, C-4, and C-5, H-1′ to C-2′ and C-5′, and H-3′ to C-2′, C-4′, and C-5′ indicated the presence of two aromatic rings. Connection between the unit **a** and the aromatic ring through C-8 were elucidated by an HMBC correlation for H-5 to C-8 and a NOESY correlation between H-5 and H_2-9. The HMBC correlation for H-1′ to C-7′ placed the unit **b** at C-6′. Connection between C-2 and C-5′ through an ether linkage was supported by the NOESY correlation between H-3 and H_2-7′. Thus, **2** was a new phenoxybenzofuran derivative and was named sakisacaulon A. The stereochemistry at C-8 may be racemic due to a weak optical rotation. The presence of a benzofuran moiety was also supported by comparing of the spectroscopic data to those of a phenoxybenzofuran **3** derived from **1** by sodium borohydride/ chloroethyl acetate.[11]

Table 1
^1H [δ_H (*J*, Hz)] and ^{13}C NMR Data (δ_C) of Sakisacaulon A (**2**) and Phenoxybenzofuran (**3**)

	δ_H		δ_C	
	2	**3**	**2**	**3**
1			108.0	105.9
2			159.9	159.0
3	6.08 (1H, d, 1.4)	6.05 (1H, d, 1.4)	102.9	100.6
4			168.9	167.1
5	6.69 (1H, d, 1.4)	6.66 (1H, d, 1.4)	101.2	98.8
6			149.7	155.2
7			167.7	169.5
8		5.47 (1H, d, 1.4)	102.6	80.8
9	2.17 (2H, m)	1.76 (2H, m)	39.7	34.0
10	2.06 (2H, m)	1.43 (2H, m)	26.8	26.4
11	1.34 (2H, m)	1.41 (2H, m)	23.6	22.1
12	0.90 (3H, t, 7.1)	0.95 (3H, t, 7.1)	14.4	13.0
1′	6.23 (1H, d, 2.8)		108.7	114.5
2′			156.7	153.3
3′	6.30 (1H, d, 2.8)	6.39 (1H, d, 2.8)	102.9	101.1
4′			151.4	147.1
5′			133.6	132.2
6′			138.2	135.3
7′	2.37 (2H, m)	2.46 (2H, m)	31.3	26.9
8′	1.51 (2H, m)	1.39 (2H, m)	30.9	28.8
9′	1.24 (2H, m)	1.22 (2H, m)	32.8	31.8
10′	1.20 (2H, m)	1.21 (2H, m)	23.4	21.9
11′	0.82 (3H, t, 7.1)	0.80 (3H, t, 7.1)	14.3	12.9
4-OMe	3.77 (3H, s)	3.77 (3H, s)	56.7	55.1
1′-Me		2.09 (3H, s)		10.0

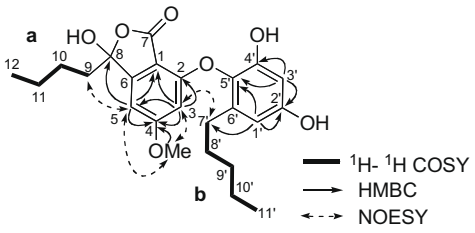

Figure 2. Selected 2D NMR correlations for Sakisacaulon A (**2**).

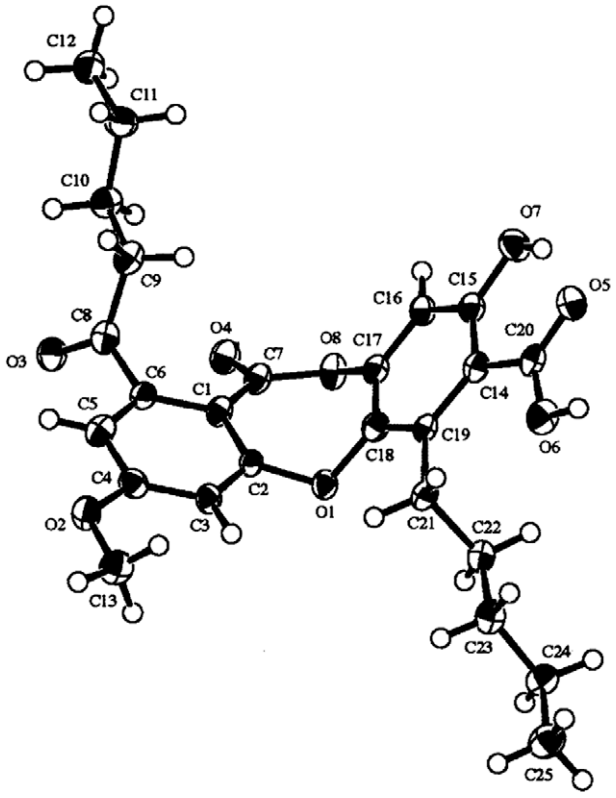

Figure 1. ORTEP drawing for lobaric acid (**1**).

In this study, it was found that lobaric acid (**1**) remarkably inhibited the polymerization of tubulin. Microtubule polymerization and depolymerization were monitored by the increase and the decrease in turbidity. Inhibitory effects of **1** to tubulin polymerization are shown in Figure 3, in which tubulin polymerization was inhibited in a concentration-dependent manner (IC_{50} 100 μM). On the other hand, sakisacaulon A (**2**) and phenoxybenzofuran (**3**) with bond cleavage of ester linkage of depsidone did not show inhibition of polymerization of tubulin. On the other hand, some derivatives of lobaric acid (**1**) such as methyl ester (**4**), acetate (**5**), methyl ester acetate (**6**), and propionate (**7**), which were prepared by treatment of **1** with trimethylsilyl diazomethane and/or acetic anhydride or propionyl chloride in pyridine, were found to be less potent than lobaric acid itself, indicating that the presence of the carboxylic acid and hydroxy at C-1′ and C-2′, respectively, is important for the activity.[12]

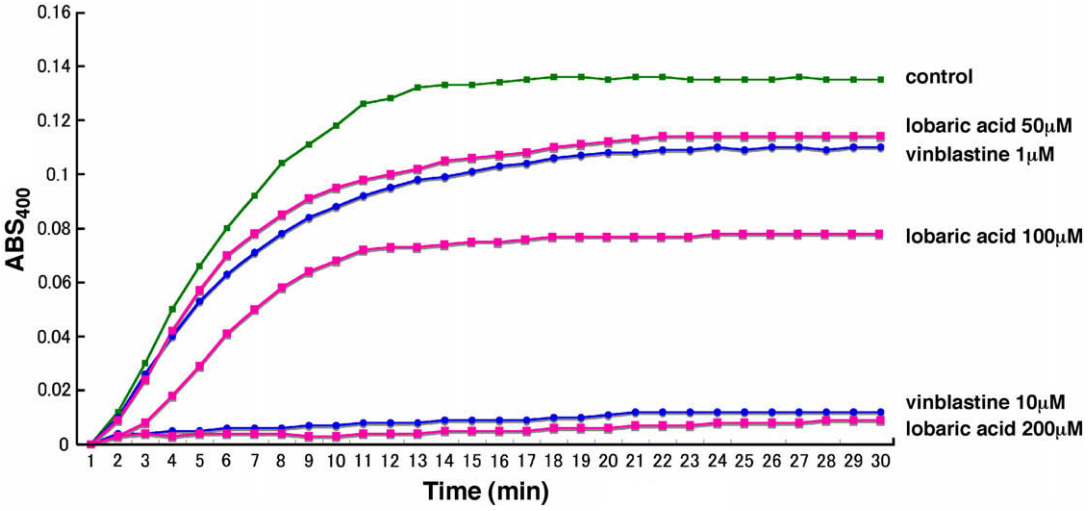

Figure 3. Inhibitory effects of lobaric acid (**1**) and vinblastine to the polymerization of tubulin protein. Various concentrations of **1** were mixed with tubulin protein (1.5 mg/mL) at 0 °C and incubated at 37 °C. The absorbance at 400 nm was measured.

Cytotoxicity of depsidone related compounds has been studied.[13] Lichen metabolites such as sphaerophorin, pannarin, induced apoptosis in human melanoma cells[14] and usnic acid was also shown to induce apoptosis of murine leukemia L1210 cells.[15] Common structural feature among these compounds is the presence of two aromatic rings which can be connected through one or two atoms bridge spacer. Orientation of the two aromatic rings is required to be ca. 125°. In addition, the appropriate torsion between two aromatic planes and the appropriate functions such as carboxylic acid and hydroxy may be important to show activity. In this work, we found that lobaric acid from lichen, *S. sasakii* showed antimitotic activity. Efforts are currently underway to determine the structure–activity relationship for antimitotic activity of a series of depsidones as antimitotic agents.

Acknowledgment

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8. All measurements were made on a Rigaku RAXIS RAPID imaging plate area detector with graphite monochromated Cu-Kα radiation. Crystal data of **1**: A colorless platelet crystal, $C_{25}H_{28}O_8$, $0.20 \times 0.08 \times 0.03$ mm, triclinic, $a = 4.73139(13)$ Å, $b = 11.3895(4)$ Å, $c = 11.4300(3)$ Å, $\alpha = 63.8883(19)°$, $\beta = 82.2266(17)°$, $\gamma = 82.561(2)°$, $V = 546.30(3)$ Å^3, $z = 1$, the calculated density is 1.387 g/cm^3. Of the 9935 reflections that were collected, 1980 were unique. The structure was solved by direct methods. $R_1 = 0.0477$ ($I > 2.00\sigma(I)$). All calculations were performed using the CrystalStructure crystallographic software package except for refinement, which was performed using SHELXL-97.
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10. Sakisacaulon A (**2**): colorless solid, $[\alpha]_D^{22}$ +4 (*c* 1.0, MeOH); IR (KBr) ν_{max} 3360, 2960, 2940, 1740, and 1620 cm^{-1}; ^{1}H and ^{13}C NMR (Table 1); ESIMS *m/z* 453 $(M+Na)^+$; HRESITOFMS *m/z* 453.1890 $(M+Na)^+$, calcd for $C_{24}H_{30}O_7Na$ 453.1889.
11. To a solution of lobaric acid (55 mg) and Et_3N (44 μL) in THF (2 mL) at 0 °C was added $ClCO_2Et$ (31 μL). After the mixture was stirred for 1.5 h at 0 °C, a solution of $NaBH_4$ (39 mg) in H_2O (2 mL) was added and stirred for 1.5 h at 0 °C, and then temperature was allowed to rise to rt. and it was stirred for an additional 2 h. The mixture was neutralized with 1 M HCl and the aqueous layer was extracted with EtOAc. Purification or the EtOAc extracts by ODS HPLC (85% MeOH) gave **3** (10 mg): colorless solid, IR (KBr) ν_{max} 3383, 2958, 1736, and 1612 cm^{-1}; ^{1}H and ^{13}C NMR (Table 1); ESIMS *m/z* 429 $(M+H)^+$; HRESITOFMS *m/z* 429.2669 $(M+H)^+$, calcd for $C_{25}H_{33}O_6$ 429.2272.
12. Compounds **2**–**7** did not show inhibition of tubulin polymerization at 200 μM.
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