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A NOVEL SECONDARY METABOLITE FROM *LETHARIELLA SERNANDERI*

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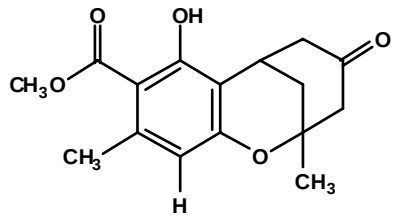
Abstract – A novel secondary metabolite, named sernanderin (**1**), was isolated from a lichen thallus, *Lethariella sernanderi*. The structure was elucidated on the basis of spectroscopic data, especially 2D-NMR spectrum and X-Ray crystallographic analyses. This compound (**1**) has a new skeletone as a natural product so far reported.

Lethariella sernanderi (Mot.) Obermayer (Usneaceae)[1] was used for a health-promoting tea in China and Tibet. Norstictic acid, atranorin, gyrophoric acid, connorstictic acid and canarione were isolated from this plant and, among the same genus, psoromic acid and gyrophoric acid were isolated from *L. cladonioides*, atranorin, psoromic acid, 2'-*O*-demethylpsoromic acid and canarione, from *L. sinensis*, and atranorin, gyrophoric acid, norstictic acid, canarione and lecanoric acid, from *L. cashmeriana*.[2]

Now we isolated a novel compound, named sernanderin, with a new skeletone as a natural product, from *Lethariella sernanderi.* The lichen thallus (1.0 kg) was extracted with ether, acetone and MeOH three times, respectively. The MeOH extract (36.9 g) was subjected to column chromatography on silica gel using $CHCl_3$-MeOH stepwise gradient system ($CHCl_3$-MeOH 100:1→50:1→20:1→10:1→5:1→2:1→1:1) to yield Fr. 1 to Fr. 8. Fr. 6 was repeatedly chromatographed on a silica gel column by using *n*-hexane-AcOEt to obtain compound (**1)** (21.0 mg).

Compound (**1)** was obtained as colorless prism (from $CHCl_3$-MeOH), mp 145.5—146.3 °C was suggested to have the molecular formula $C_{16}H_{18}O_5$ by its positive HRFABMS spectral data *m/z* 291.1223 $[M+H]^+$,



Sernanderin (**1**)

(calcd 291. 1232, $C_{16}H_{19}O_5$, $[M+H]^+$). The UV spectrum ($CHCl_3$) of **1** showed absorption maxima at λ_{max} (log ε): 240 (3.94), 269 (4.29), 303 (3.89). The IR spectrum exhibited presences of a hydroxyl (3420 cm^{-1}) and carbonyl (1720, 1642 cm^{-1}) groups. The structure of **1**, a racemic compound, was determined by X-Ray crystallographic analysis[3] (Figure 1). The ^{1}H-NMR spectrum (Table 1) showed signals due to three singlet methyls at δ 1.52, 2.41 and 3.90 (each 3H, s). The second methyl group was considered to attach on sp^2 carbon, from the chemical shift and third methyl group was considered to methoxy methyl or methyl ester. The ^{13}C-NMR spectrum (Table 1) revealed 16 carbon signals, which were sorted by DEPT as three methyls, three methylenes, two methines and eight quaternary carbons including one carboxyl and one carbonyl function. Based on combination of the HMQC, ^2J and ^3J HMBC and DQF-COSY experiments, the structure was determined and allowed assignments of all protons and carbons. The methine proton at δ 6.14 having HMQC correration at δ 111.5 methine carbon, showed HMBC correlation with the aromatic quaternary carbons at δ 104.6, 109.4, 141.3 and 157.0. The HMBC correlation, between the methyl protons at δ 3.90 and the carbonyl carbon at δ 172.4, suggested the presence of carboxymethyl group. The hydroxy proton at δ 12.07 showed HMBC correlation with aromatic quaternary carbons at δ 104.6, 109.4 and 161.8. The methyl protons at δ 2.41 showed HMBC correlation with the methine carbon at δ 111.5 and aromatic quaternary carbons at δ 104.6 and 141.3. The methyl protons at δ 1.52 had cross peaks with δ 34.9 (CH_2), 53.4 (CH_2) and 77.6 (C) on HMBC. The methylene protons at δ 2.43 or 2.65, showing HMQC correlation with methylene carbon at δ 53.4, showed HMBC correlation with the carbonyl carbon at δ 208.2, the quaternary carbon at δ 77.6, the methylene carbons at δ 34.9 and 45.9, and the methyl carbon at δ 28.7. The methylene protons at δ 2.54 or 2.75, showing HMQC correlation with methylene carbon at δ 45.9, showed HMBC correlation with the carbonyl carbon at δ 208.2, the methylene carbons at δ 34.9 and 53.4, methine carbon at δ 26.5 and aromatic quaternary carbon at δ 109.4. The methylene protons at δ 2.09 or 2.26, showing HMQC

correlation with methylene carbon at δ 34.9, showed HMBC correlation with the quaternary carbon at δ 77.6, the methylene carbons at δ 45.9 and 53.4 methine carbon at δ 26.5 and aromatic quaternary carbon at δ 109.4. The methine proton at δ 3.67, showing HMQC correlation with methine carbon at δ 26.5, showed HMBC correlation with the quaternary carbon at δ 77.6 and carbonyl carbon at δ 208.2, respectively. From these HMBC correlation (Figure 2), the structure of compound (**1**) was suported and umbigous assignment of ^{1}H- and ^{13}C-NMR of **1** were shown in Table 1. Therefore, the structure of sernanderin (**1**) was determined to methyl 7-hydroxy-2,9-dimethyl-2,3,5,6-tetrahydro-2,6-methano-4*H*-1-benzoxocin-4-one- 8-carboxylate as shown in **1**.

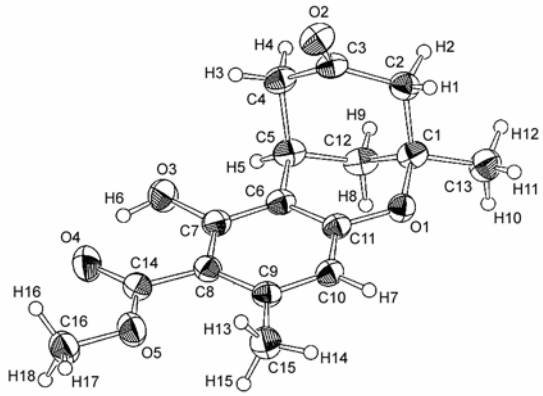


Figure 1. ORTEP drawing of **1**

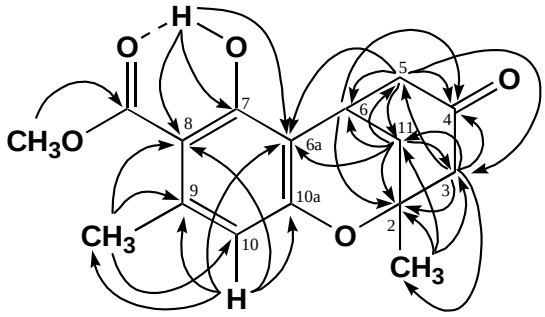


Figure 2. HMBC correlation of **1**

Table 1. ^{13}C- and ^{1}H-NMR spectral data of **1** in $CDCl_3$

Position	$^{\delta}$C	$^{\delta}$H (mult., *J* in Hz)
2	77.6	
3	53.4	2.43 (1H, d, 16.5), 2.65 (1H, dt, 16.5, 2.4)
4	208.2	
5	45.9	2.54 (1H, dd,15.1, 4.3), 2.75 (1H, dq, 15.1, 2.4)
6	26.5	3.67 (1H, m)
6a	109.4	
7	161.8	
8	104.6	
9	141.3	
10	111.5	6.14 (1H, s)
10a	157.0	
11	34.9	2.09 (1H, m), 2.26 (1H, dd, 13.7, 2.4)
2-CH_3	28.7	1.52 (3H, s)
9-CH_3	24.1	2.41 (3H, s)
8-$\underline{C}OOCH_3$	172.4	
8-$COO\underline{CH_3}$	51.8	3.90 (3H, s)
7-OH		12.07 (1H, s)

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REFERENCE AND NOTES

1. *Lethariella sernanderi* (Mot.) Obermayer (Usneaceae) was identified by the late Professor William Lowis Culberson. A voucher specimen is deposited at the Department of Pharmacognosy and Phytochemistry, Meiji Pharmaceutical University.
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3. X-Ray crystallographic analysis: All measurements were made on a Rigaku AFC7S diffractometer with graphite monochromated Cu-Kα radiation (λ = 1.5418 Å). Crystal data: Colorless prismatic crystal, monoclinic, $C_{16}H_{18}O_5$ (*Mr* = 290.32), space group $P2_1/n$ with *a* = 18.316(1) Å, *b* = 9.9495(6) Å, *c* = 8.0536(4) Å, β = 99.086(5)°, *V* = 1449.2(2) Å^3, *Z* = 4, and D_{calcd} = 1.330 gcm^{-3}. The structure was solved by direct methods (SIR97[4]) and expanded using Fourier techniques (DIRDIF94[5]). The non-hydrogen atoms were refined anisotropically. Hydrogen atoms were included but not refined. The final cycle of full-matrix east-squares refinement was based on 2603 all reflections (2θ < 135.98°) and 191 variable parameters and converged with unweighted and weighted agreement factors of R = 0.055, R_w = 0.217 and R_1 = 0.042 for $I > 2.0\sigma(I)$ data.
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