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Isolation, Structural Determination, and Total Synthesis of A New Biologically Active δ-Lactone Produced by *Seiridium unicorne*

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Abstract: The structure of a new biologically active δ-lactone, produced by *Seiridium unicorne*, was determined to be (2*S*,3*R*,5*S*)-(-)-2,3-dihydroxytetradecan-5-olide. The relative configuration was elucidated from NMR experiments. The synthesis of the enantiomer from D-glucose revealed the absolute configuration. The total synthesis of the natural form was also achieved from (*R*)-malic acid.

In our series of studies on tree disease, we have already reported aliphatic δ-lactones produced by *Ceratocytis piceae*[1] and abscisterols produced by *Cryptosporiopsis abietina*.[2] The resinous canker disease of *Chamaecyparis obtusa* Sieb. et Zucc (Hinoki) occurs mainly in young trees. The pathogen of this disease was identified to be *Seiridium unicorne* by Yamada.[3] In this paper, we describe the isolation, structural determination, and total synthesis of a new biologically active δ-lactone from *S. unicorne*.

S. unicorne was cultivated on 3% potato-glucose medium (total 24 l) at 25 ℃ for 4 weeks in the dark. The mycelia obtained by filtration were homogenized and extracted with acetone. After evaporation of acetone, the resulting residue was partitioned between water and EtOAc. Evaporation of the EtOAc layer gave a residue (11.8 g) which was separated into hexane soluble and insoluble fractions. The residue (8.9 g) obtained from the hexane soluble fraction was repeatedly chromatographed on silica gel with several solvent-systems [(1) $CHCl_3$-MeOH gradient elution; (2) hexane-EtOAc gradient elution, (3) hexane:EtOAc = 1:1, (4) $CHCl_3$:MeOH = 97:3, (5) $CHCl_3$:acetone = 8:2, and (6) $CHCl_3$:MeOH = 97:3]. Each fraction was assayed with the guidance of the abscisic activity against the leaves of *C. obtusa*,[2a,c] the growth inhibition against lettuce seedlings, and the antifungal activity against *Cladosporium herbarum*.[1] A new compound (**1**) [Fig. 1], which exhibited positive activities in all three assay-systems, was finally isolated as a white powder (8.5 mg); mp 96-100 ℃; $[\alpha]_D^{24}$ -35.0° (*c* 0.33, $CHCl_3$); IR ν_{max}(KBr) cm^{-1}: 3422 (OH), 2921, 2852, 1720 (C=O), 1467, 1233 (CO-O).

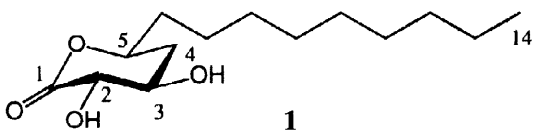

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Fig. 1. The structure of a new biologically active δ-lactone (**1**) isolated from *S. unicorne*

The molecular formula of **1** was determined as $C_{14}H_{26}O_4$ from HR-EI-MS [calcd. for $C_{14}H_{27}O_4$ (MH^+): m/z 259.1909; found 259.1907]. The ^{1}H- and ^{13}C-NMR spectra of **1** are shown in Table 1. One quaternary carbon (δ 173.4 ppm), three oxy-methine groups, nine methylene groups, and one methyl group were observed

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in the ^{13}C-NMR and DEPT spectra. By considering the degree of unsaturation (= 2) and the IR spectrum, **1** would be a dihydroxy-δ-lactone possessing a nonyl group. As we expected, acetylation of **1** gave diacetate **2** (Table 1 and Fig. 2) which was mainly used for structural analysis. In the ^{1}H-NMR spectrum of **2**, two acetoxy-methine protons were newly observed at δ 5.05 (d, H-2) and δ 5.35 (ddd, H-3) with downfield shift while one oxy-methine proton at δ 4.40 (m, H-5) was rarely shifted in comparison with that of **1**. The partial structure (C-2~C-5) of **2** was elucidated from the ^{1}H-^{1}H-COSY spectrum. By considering the multiplicity of H-2 and H-5, the plane structure of **2** was determined as 2,3-diacetoxy-5-nonyl-δ-lactone. Coupling constants [H-2/3 (9.6 Hz), H-3/4$_{ax.}$ (11.9 Hz) and H-4$_{ax.}$/5 (11.2 Hz)] and NOE enhancements [H-2/4$_{ax.}$ and H-3/5] are all explicable to be axial orientation of H-2, H-3 and H-5. Therefore, the relative structure of **1** was determined as (2*S**,3*R**,5*S**)-2,3-dihydroxytetradecan-5-olide.

Table 1. ^{13}C-NMR (67.5 MHz) and ^{1}H-NMR (270 MHz) data of **1** and **2** in $CDCl_3$[a]

	1		**2**	
C/H	δ_C ppm	δ_H ppm	δ_C ppm	δ_H ppm
1	173.4 (s)		166.5 (s)	
2	74.2 (d)	3.97 (1H, br. d, *J* = 9.9 Hz)	71.6 (d)	5.05 (1H, d, *J* = 9.6 Hz)
3	68.7 (d)	4.05 (1H, m)	68.9 (d)	5.35 (1H, ddd, *J* = 11.9, 9.6, 4.6 H)
4	36.0 (t)	2.26 (1H, dt, *J* = 13.9, 3.3 Hz)	35.2 (t)	2.38 (1H, ddd, *J* = 13.5, 4.6, 2.3 Hz)
		1.81 (1H, ddd, *J* = 13.9, 11.9, 11.2 Hz)		1.81 (1H, ddd, *J* = 13.5, 11.9, 11.2 Hz)
5	78.4 (d)	4.32 (1H, m)	77.8 (d)	4.40 (1H, m)
6	35.7 (d)	1.72 (1H, m)	34.2 (d)	1.74 (1H, m)
		1.63 (1H, m)		1.63 (1H, m)
7	24.8 (t)	1.47 (1H, m)	24.6 (t)	1.47 (1H, m)
		1.37 (1H, m)		1.37 (1H, m)
8	29.5 (t)[b]		29.0 (t)[b]	
9	29.4 (t)[b]		29.0 (t)[b]	
10	29.3 (t)[b]		29.0 (t)[b]	
11	29.3 (t)[b]	1.26 (2H x 6, s)[c]	29.0 (t)[b]	1.26 (2H x 6, s)[c]
12	31.8 (t)		31.8 (t)	
13	22.6 (t)		22.6 (t)	
14	14.1 (q)	0.88 (3H, t, *J* = 6.6 Hz)	14.1 (q)	0.88 (3H, t, *J* = 6.6 Hz)
OH		dependence on the concentration	OAc	170.0 (s) x 2 2.17 (3H, s)
		(1H, br. s) x 2		21.0 (q) x 2 2.07 (3H, s)

a. Assignments were based on DEPT, ^{1}H-^{1}H-COSY, and ^{1}H-^{13}C-COSY spectra.
b. ^{13}C-signals (C-8, -9, -10 and -11) are indistinguishable and exchangeable.
c. ^{1}H-signals (H-8, -9, -10, -11, -12 and -13) are overlapped.

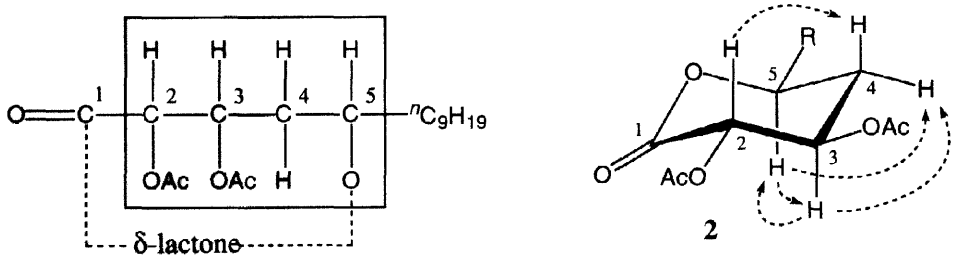
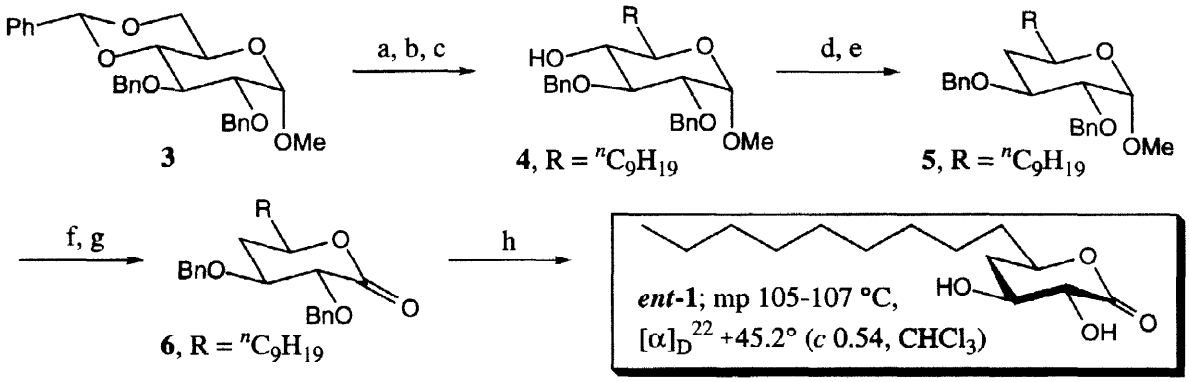


Fig. 2. Partial structure of diacetate **2** elucidated from ^{1}H-^{1}H-COSY and NOE enhancements

The absolute configuration of **1** was determined by the synthesis of ***ent*-1** from D-glucose (Scheme 1) because both **1** and D-glucose have the same relative configuration with respect to C-2,3,5 stereogenic centers.

Three modifications, (1) C_8-elongation at the C-6 position, (2) deoxygenation at the C-4 position, and (3) oxidation at the C-1 position, are required for D-glucose. Known compound **3** obtained from D-glucose in 3 steps[4] was deprotected to give a diol (C-4,6)[5] whose primary hydroxyl group was selectively tosylated with tosyl chloride and pyridine. The resulting tosylate was elongated to give **4** with Grignard reagent [$CH_3(CH_2)_7MgBr$] in the presence of copper (I) salt. The secondary hydroxyl group of **4** was deoxygenated to give **5** *via* chlorination with triphenylphosphine in refluxing carbon tetrachloride followed by radical reduction with tributyltin hydride. Acidic hydrolysis of **5** and subsequent oxidation with pyridinium dichromate gave δ-lactone **6**. Two benzyl groups of **6** were deprotected by hydrogenolysis to give ***ent*-1** as a white powder, whose spectral data (^{1}H-, ^{13}C-NMR, IR, MS) were completely identical with those of natural **1**, except for the sign of the specific rotation.[6] Therefore, the absolute structure of **1** was unambiguously determined as (2*S*,3*R*,5*S*)-(-)-2,3-dihydroxytetradecan-5-olide.

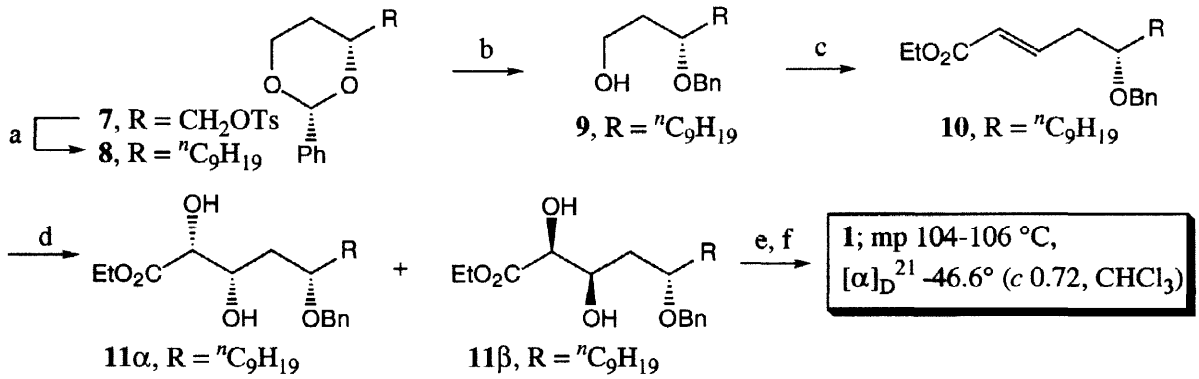


Scheme 1: (a) TsOH / MeOH, 100%; (b) TsCl, pyridine / CH_2Cl_2, 95%; (c) $CH_3(CH_2)_7MgBr$, CuI / THF, 77%; (d) Ph_3P / CCl_4, 100%; (e) *n*-Bu_3SnH, AIBN / toluene, 92%; (f) 12 M H_2SO_4-dioxane (1:3), 95%; (g) PDC, MS 4A / CH_2Cl_2, 81%; (h) H_2, $Pd(OH)_2$ / EtOAc, 81%.

Furthermore, the total synthesis of **1** *via* a new route was planned to investigate in detail its biological activity (Scheme 2). (*R*)-Malic acid was used as a chiral starting material because L-glucose is too expensive to use. Known tosylate **7** obtained from (*R*)-malic acid in 3 steps[7] was elongated to give **8** in 97% yield by the same method as used in the synthesis of ***ent*-1**. Reductive cleavage of benzylidene acetal **8** with diisobutylaluminum hydride[4b, 8] proceeded regioselectively at the sterically less-hindered primary site. Primary alcohol **9** was obtained in 99% yield as the sole product. Swern oxidation of **9** and subsequent Wittig reaction with (carbethoxymethylene)triphenylphosphorane in one-pot gave (*E*)-unsaturated ester **10** in 85% yield along with 10% of (*Z*)-isomer. Both isomers could be readily separated by silica-gel column chromatography. Osmium oxidation of **10** without a chiral ligand was carried out to examine the intrinsic diastereofacial selectivity prior to Sharpless AD reaction.[9] The reaction of **10** with a catalytic amount of osmium tetraoxide and *N*-methylmorpholine *N*-oxide as a cooxidant in aqueous acetone gave an inseparable 1:1 mixture of diols **11**α (undesired) and **11**β (desired). This result suggests that the reagent-control would be possible for **10** under Sharpless AD conditions. In practice, the highly diastereoselective reaction with AD-mix-β proceeded to give a 1:21 mixture of **11**α and **11**β in 100% yield (91% *d.e.*). The ratio of **11**α and **11**β was determined from the integration of ^{1}H-NMR. Hydrogenolysis of the mixture of **11**α and **11**β (91% *d.e.*) gave a mixture of the corresponding triols and **1** which was treated with acetic acid in refluxing benzene to promote lactonization. After the completion of lactonization, the reaction mixture was evaporated to dryness. The resulting powdery

residue was reprecipitated from hexane/EtOAc to give **1** in 81% yield as a white powder, whose spectral data (^{1}H-, ^{13}C-NMR, IR, MS) were completely identical with those of natural **1**. The sign of the specific rotation of synthetic **1** was also similar to that of natural **1**.[6] The diastereomer derived from **11**α was completely removed by recrystallization.

While synthetic **1** as well as natural **1** exhibited the abscisic activity against the leaves of *C. obtusa*[2a] at the same concentrations with the evolution of typical Hinoki odor, ***ent*-1** exhibited no activity. The minimum effective concentration (25 ppm) of **1** is higher than that of abscisterol A.[2] The total synthesis of **1** made it possible to examine the influence of **1** on the resinous canker disease of *C.* obtusa. Other biological activities of **1** and ***ent*-1** are now under examination and will be reported elsewhere.



Scheme 2: (a) $CH_3(CH_2)_7MgBr$, CuI / THF, 97%; (b) DIBAL-H / CH_2Cl_2, 99%; (c) Swern oxid. then $Ph_3P{=}CHCO_2Et$, 85%; (d) AD-mix-β, $MeSO_2NH_2$ / *t*-BuOH-H_2O (1:1), 100%, 91% *d.e.*; (e) H_2, $Pd(OH)_2$ / EtOH; (f) AcOH / benzene, 81%

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References and Notes

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5. Satisfactory spectral data (^{1}H-NMR, ^{13}C-NMR, IR, HRMS, $[\alpha]_D$) were obtained for all new compounds.
6. The mp and $[\alpha]_D$ of synthetic compounds (**1** and ***ent*-1**) were higher and larger than those of natural **1** because natural **1** was slightly contaminated with an artifact, methyl 2,3,5-trihydroxytetradecanoate. Methanol used in chromatography is responsible for the opening of the lactone-ring. Hydrogenolysis of **6** in methanol also gave a mixture of ***ent*-1** and methyl 2,3,5-trihydroxytetradecanoate.
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