Note

Structure of Sescandelin-B, a New Metabolite Produced by the Fungus, *Sesquicillium candelabrum*

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Sescandelin (**1**)¹⁾ has been isolated from the culture filtrate of *Sesquicillium candelabrum* IFO 30556 as a root-promoting substance which acts synergistically with IAA. In the course of our further search for new root-promoting substances from this fungus, we obtained a new compound named sescandelin-B (**2**) which did not show biologically activity itself. However, triacetyl sescandelin-B (**3**) at 100 $\mu\text{g/ml}$ promoted root formation in *Azukia* cuttings²⁾ with 4 days of cultivation by about 40% over the control when IAA was applied on the second day, after applying **3** on the first day. In this paper, we describe the isolation and structural elucidation of this new compound **2**.

The fungus was statically cultured in a malt extract medium, the culture filtrate being treated with active charcoal at pH 2.0, and successively extracted with acetone. The extract was concentrated and the residue was chromatographed on a silica gel column (benzene–acetone). The eluate with benzene–acetone (9:1) gave a mixture of **1** and **2**. **2** was obtained by medium-pressure column chromatography on Kiesel 60 HF₂₅₄ gel eluting with benzene–acetone (9:1 and 85:15), and by preparative TLC on Kiesel 60 HF₂₅₄ gel, developing with benzene–acetone (8:2), in a yield of 3 mg/l.

The physicochemical properties of **2** were colorless plates (from MeOH), mp 248–250°C; UV λ_{max} (EtOH) nm (ϵ): 200 (8500), 237 (15,000), 244 (17,000), 259 (sh, 7800), 277 (sh, 6100), 290 (sh, 4100), 317 (2300); IR ν_{max} (KBr) cm^{-1} : 3430, 3210, 1690, 1650, 1630, 1605, 1590, 1250, 1180; EIMS m/z (%): 222 [M^+] (100), 204 (50), 180 (68), 150 (100). The molecular formula was determined by MS and elementary analysis (Found: C, 59.38; H, 4.46. Calcd. for $\text{C}_{17}\text{H}_{16}\text{O}_5$: C, 59.46; H, 4.54%). A positive FeCl_3 test together with the IR (3210 cm^{-1}) and ^1H -NMR spectra (in acetone- d_6 , δ_{H} 11.16) indicated the presence of a chelated phenolic hydroxyl group. The signals at δ_{H} 6.62 and 6.94 ($J=2.0$ Hz) revealed the presence of a pair of *meta*-coupling protons on a benzene ring. The vinyl methyl and hydroxymethyl protons appeared at δ_{H} 2.24 (3H, s) and 4.69 (2H, s). The presence of a $-\text{COO}-$ group was suggested by the IR band at 1690 cm^{-1} and the ^{13}C signal at δ_{C} 166.3. The UV spectrum of **2** was very similar to that of 6,8-dihydroxyisocoumarin³⁾ and superimposable on that of **1**.

By acetylation with Ac_2O –pyridine, **2** yielded a triacetyl sescandelin-B (**3**), as colorless needles (from acetone), mp 125–128°C; UV λ_{max} (EtOH) nm (ϵ): 235 (44,300), 259 (18,100), 266 (18,600), 277 (sh, 15,000), 320 (5600); IR ν_{max} (KBr) cm^{-1} : 2950, 1790, 1660, 1620, 1580, 1380, 1250, 1200; HRMS m/z : 348.0850 (M^+ , 348.0914 calcd. for $\text{C}_{17}\text{H}_{16}\text{O}_8$). In the ^1H -NMR spectrum of **3**, the downfield shifts of a pair of *meta*-coupling protons of **2** (from δ_{H} 6.62 and 6.94 to 6.94 and 7.21, respectively) indicated the phenolic hydroxyl groups being adjacent to those protons.

Catalytic hydrogenation over PtO_2 of **2** gave a derivative (**4**) as colorless crystals, mp 194–196°C; UV λ_{max} (EtOH) nm (ϵ): 200 (39,000), 205 (sh, 34,800), 214 (sh, 27,700), 228 (sh, 18,200), 266 (47,000); IR ν_{max} (KBr) cm^{-1} : 3200, 1650, 1590, 1480, 1400, 1290, 1260, 1200, 1180; HRMS m/z : 208.0754 (M^+ , 208.0736 calcd. for $\text{C}_{11}\text{H}_{12}\text{O}_4$).

The ^1H -NMR spectrum showed the presence of two methyl groups [δ_{H} 1.27 (3H, d, $J=6.0$ Hz), 1.48 (3H, d, $J=6.0$ Hz)], and two methine protons [δ_{H} 2.99 (1H, dq, $J=6.0, 3.0$ Hz), 4.77 (1H, dq, $J=6.0, 3.0$ Hz)] newly appeared. Furthermore, the signal at δ_{H} 4.77 was coupled to those at δ_{H} 1.48 and 2.99, and the signal at δ_{H} 2.99 to that at 1.27 by decoupling experiments. From these results, **4** had a partial structure of $-\text{O}-\text{CH}(\text{CH}_3)-\text{CH}(\text{CH}_3)-$. Since the ^1H -NMR spectrum [δ_{H} 2.24, (3H, s); 4.69, (2H, s)] of **2** showed the presence of vinyl methyl and hydroxymethyl groups, one of the two methyls in **4** must

Table I. PROTON AND CARBON NMR
DATA FOR SESCANDELIN-B (**2**)

Carbon no.	δ_H	δ_C
1		166.3
3		152.7
4		114.1
4a		140.8
5	6.94 d (2.0) ^a	102.6
6		167.5
7	6.62 d (2.0) ^a	102.3
8		164.6
8a		99.7
9	2.24 s	16.7
10	4.69 s	57.2
OH	11.16 s	

¹³C (67.80 MHz) and ¹H (270.05 MHz) in CD₃COCD₃.^a Coupling constants in Hz.

have been derived from a hydroxymethyl group bonded to a double bond in **2** by hydrogenolysis.⁴⁾

In the NOE difference spectrum of **3**, irradiation of the signal at δ_H 7.21 due to an aromatic proton gave rise to an enhanced signal at δ_H 5.09 due to methylene protons, but not to that at δ_H 2.39 due to methyl protons. Also,

irradiation of the methylene protons at δ_H 5.09 (C₁₀) caused NOE of the resonance at δ_H 2.39 (C₉) and 7.21 (C₅).

The signals of the ¹H- and ¹³C-NMR spectra of **2** were correlated by selective proton decoupling experiments and by comparing with those of **1**.¹⁾ Thus, the structure of **2** was elucidated to be 6,8-dihydroxy-4-hydroxymethyl-3-methyl-isocoumarin.

The structure-activity relationship among **1**, **2** and their derivatives to *Azukia* cuttings, together with biosyntheses of **1** and **2** will be reported in the near future.

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