**Note**

**Biosynthesis of Resorcylic Acid Lactone (5S)-5-Hydroxyasiodiplodin in Lasiodiplodia theobromae**

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An administration study of 2H-labeled precursors showed that the 9-hydroxydecanoyl unit, the acyl intermediate of lasiodiplodin (1), was also the intermediate of lasiodiplodin (6) into 2 indicated that hydroxylation at C-5 occurred after cyclization.

Key words: lasiodiplodin; biosynthesis; resorcylic acid lactone; Lasiodiplodia theobromae

Lasiodiplodins are unique fungal metabolites of *Lasiodiplodia theobromae* which possess various physiological activities. They are classified as resorcylic acid lactones (RALs). The skeletons of RALs are mainly biosynthesized by two polyketide synthases (PKSs), reducing PKS (R-PKS) and non-reducing PKS (NR-PKS), via highly reduced acyl intermediates. In our own biosynthetic study on polyketides produced by *L. theobromae*, we have demonstrated that lasiodiplodin 1, an RAL, was also biosynthesized via the typical biosynthetic system of RALs. However, the biosynthetic pathway for the hydroxylated derivative of 1, (5S)-5-hydroxyasiodiplodin (2), has not yet been revealed, especially hydroxylation at C-5. We here report further labeling studies indicating that 1 was biosynthesized via direct hydroxylation of 2.

(1H, = 6.45 (1H, d, J = 2.3 Hz), 6.45 (1H, d, J = 2.3 Hz), 5.17 (1H, m), 2.31 (1H, m), 2.51 (1H, m), 1.23 (3H, s), 1.96-1.66 (2H, m), 1.67-1.50 (2H, m), 1.54-1.35 (6H, m), 1.31 (3H, s); 13C-NMR, CDC13 (δ13C = 77.0). 10,10,10-2H3)-9-hydroxydecanoic acid (3) and acetyl-lasiodiplodin (4). These were prepared as previously reported.

Acetyl-de-O-methyl-lasiodiplodin (5). To a solution of 4 (821 mg, 2.46 mmol) in dry CHCl3 (40 ml), a 1.0 M BBr3 in CHCl3 solution (19.0 ml) was added dropwise at -78°C. The mixture was stirred at -78°C for 4 h and poured into ice-cooled water. The organic layer was separated and the aqueous layer was extracted with CH2Cl2 (100 ml × 3). The combined organic layers were washed with brine, dried over MgSO4, and evaporated to dryness. The residue was purified by silica gel column chromatography (silica gel 40 g; EtOAc:n-hexane = 10:90, v/v) to give 190 mg of 5 as a colorless oil in a 24% yield. HRFD-MS m/z: 320.1609 [M+Na]++ (calcd. for C18H18O4, 320.1624); 1H-NMR (270MHz, CDCl3) δ (ppm): 11.6 (1H), 5.65 (1H, d, J = 2.3 Hz), 6.45 (1H, d, J = 2.3 Hz), 5.17 (1H, m), 3.26 (1H, m), 2.51 (1H, m), 1.73 (3H, s), 1.96-1.66 (2H, m), 1.67-1.50 (2H, m), 1.51-1.35 (6H, m), 1.31 (3H, s); 13C-NMR (67.5MHz, CDCl3) δ (ppm): 171.3, 168.4, 164.2, 164.4, 154.4, 154.8, 115.7, 109.9, 108.3, 75.4, 33.3, 30.9, 30.6, 27.0, 24.4, 24.0, 21.0, 20.9, 19.8.

(5O-methyl-2H3)-l-asiodiplodin (6). To a stirred solution of 5 (240 mg, 0.749 mmol) in acetonitrile (6 ml), CD13 (70.8 μl, 1.12 mmol) and then K2CO3 (129 mg, 1.12 mmol) were added at room temperature, and the mixture stirred overnight. EtOH (8 ml) and a 1.0 M NaOH aqueous solution (4 ml) were added to the solution, and the mixture stirred for an additional 3 h. The mixture was quenched with 1.0 M HCl (20 ml) and extracted with EtOAc (40 ml × 3). The combined extracts were washed with brine, dried over Na2SO4, and evaporated to

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dryness. The residue was purified by silica gel column chromatography (silica gel 40 g; EtOAc: n-hexane = 20:80, v/v) to give 122 mg of 6 as a white powder in a 55% yield. HRFD-MS \( m/z = [M]^{+} \) (calcd. for C_{17}H_{21}D_{3}O_{4}, 295.1860); \(^1\)H-NMR (270 MHz, CDCl\(_3\)) /C\(_{14}\) (ppm): 6.20 (1H, d, J = 2.3 Hz), 6.18 (1H, d, J = 2.3 Hz), 5.26 (1H, m), 2.63 (1H, m), 2.44 (1H, m), 1.89 (1H, m), 1.70–1.53 (4H, m), 1.48–1.30 (4H, m), 1.30 (3H, d, J = 6.6 Hz), 1.28–1.19 (4H, m); \(^2\)H-NMR (76.8 MHz, CHCl\(_3\)) \( \delta \) (ppm): 3.71; \(^{13}\)C-NMR (67.5 MHz, CDCl\(_3\)) \( \delta \) (ppm): 169.1, 158.0, 157.6, 143.0, 117.3, 108.3, 97.0, 72.4, 32.3, 30.4, 30.0, 26.4, 25.4, 24.1, 21.3, 19.5.

**Culture conditions.** The culture method for \( L. \) theobromae IFO 31059 and the administration method for the \(^2\)H-labeled precursors have been described in a previous report.\(^{12}\) The concentrations of 3 and 6 administered were 1 and 5 \( \mu \)M, respectively.

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\(^{1}\)H-NMR Spectra of Biosynthetically Labeled 2 from 3 (A) and from 6 (B), and \(^1\)H-NMR Spectrum of Natural 2 (C). A and B: 76.8 MHz, CHCl\(_3\). C: 500 MHz, CDCl\(_3\).

**Fig. 2.**

**Fig. 3.** Preparation of 6.

1. Ac\(_2\)O, pyridine, r.t., overnight.
2. BBr\(_3\), CH\(_2\)Cl\(_2\), -78°C, 4 h.
3. CD\(_3\)I, K\(_2\)CO\(_3\), acetone, r.t., overnight.
4. NaOH, EtOH-H\(_2\)O (2:1), r.t., 3 h.
Incorporation ratio. The incorporation ratio was calculated from the FD-MS data by comparing the [M + 3]⁺ ion peak (m/z = 311) of the natural and biosynthetically labeled compounds of 2.

Isolation of biosynthesized 2. The isolation method for 2 from the culture has been described in a previous report. Compound 2 derived from 3 (6.5 mg) and compound 2 derived from 6 (3.8 mg) were respectively obtained from 150 ml of the culture of *L. theobromae*.

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References