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Masaki Asai, Yasunao Hattori, Hidefumi Makabe

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Synthesis of legioliulin, a fluorescent isocoumarin compound, isolated from *Legionella dumoffii* using cyclic acylpalladation and Heck reaction

Masaki Asai,^a Yasunao Hattori,^b Hidefumi Makabe^{*a}

^aGraduate School of Science and Technology, Department of Agriculture, Division of Food Science and Biotechnology, Shinshu University, 8304 Minami-minowa, Kami-ina, Nagano, 399-4598, Japan

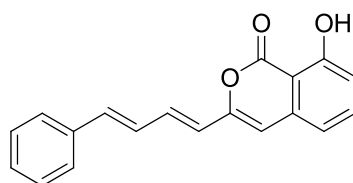
^bCenter for Instrumental Analysis, Kyoto Pharmaceutical University, Yamashina-ku, Kyoto 607-8412, Japan

*Corresponding author. Tel. +81 265 77 1630; fax +81 265 77 1700; e-mail: makabeh@shinshu-u.ac.jp

Abstract: Concise synthesis of legioliulin, an isocoumarin compound isolated from *Legionella dumoffii*, was achieved. Isocoumarin ring of legioliulin was constructed using cyclic acylpalladation. Chain elongation was performed using Heck reaction using *t*-butylphosphine as a ligand.

Key words: natural product, polyketides, isocoumarin, acylpalladation, Heck reaction

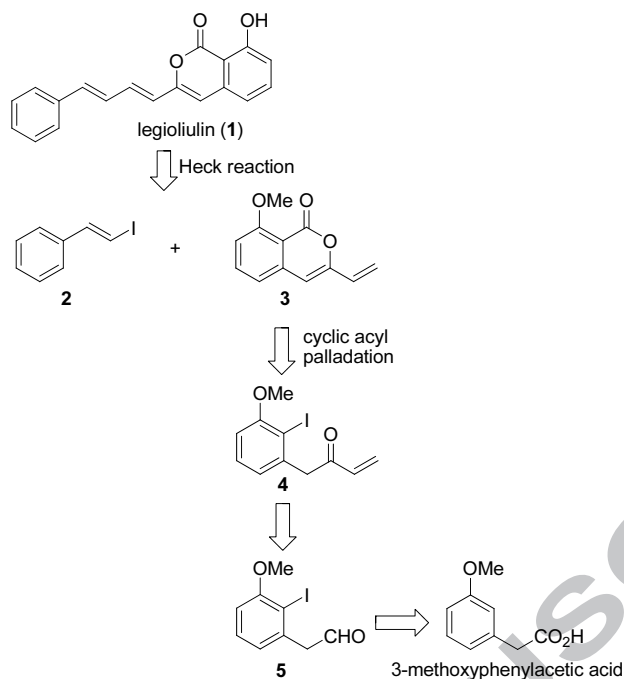
Legionellae, which are responsible for Legionnaire's disease, are facultative intracellular gram-negative bacteria.¹ 10 species of *Legionella* exhibit blue-white and dark-red autofluorescence. In 2004, Amemura-Maekawa and co-workers isolated legioliulin (**1**) from *Legionella dumoffii*.² Legioliulin (**1**) is a new isocoumarin compound and fluorescent substance. The study on biosynthesis of legioliulin (**1**) was reported by Bode and co-workers in 2013.³ This compound did not show any cytotoxicity against human monocytic cell line U937, neither exhibit antimicrobial activity against *Staphylococcus aureus* and *E. coli*.² The real function and the detailed biological activity of legioliulin (**1**) are still unknown. Thus we began to synthesize legioliulin (**1**) to find out its function and biological properties. Here, we wish to report the concise synthesis of legioliulin (**1**) using cyclic acylpalladation and Heck reaction as the key steps (Figure 1).



legioliulin (**1**)

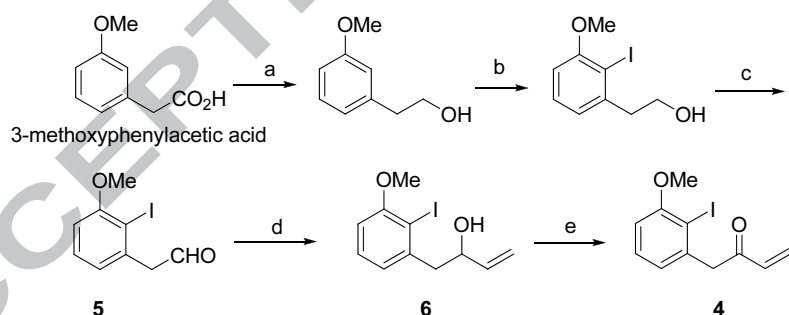
Figure 1. The structure of legioliulin (**1**).

The synthetic strategy shows in Scheme 1. The side chain would be introduced using Heck reaction. The isocoumarin part of **1** would be constructed using cyclic acyl palladation trapping by *O*-enolate from iodoenone **4**. Iodoenone **4** would be prepared from known aldehyde **5**.⁴ Aldehyde **5** would be synthesized from commercially available 3-methoxyphenylacetic acid (Scheme 1).



Scheme 1. Synthetic strategy of boronolide (1) and deacetylboronolide (2).

Scheme 2 shows the preparation of cyclization precursor 4. Reduction of carboxylic acid of 3-methoxyphenylacetic acid with LiAlH_4 afforded corresponding alcohol subsequent iodination at *ortho* position and oxidation of primary alcohol gave aldehyde 5. The yield of iodination was rather low because this compound was unstable. Alkylation of 5 using vinylmagnesium bromide afforded allylic alcohol 6. Oxidation of the secondary hydroxy group of 6 with Dess-Martin periodinane in the presence of pyridine gave enone 4 (Scheme 2).

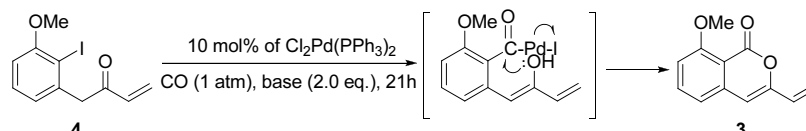


Scheme 2. Synthesis of cyclization precursor 4. Reagents and conditions: (a) LiAlH_4 , THF, 96%; (b) $n\text{-BuLi}$, I_2 , Et_2O , 48%; (c) Dess-Martin periodinane, CH_2Cl_2 , 99%; (d) vinylmagnesium bromide, Et_2O , 89%; (e) Dess-Martin periodinane, pyridine, CH_2Cl_2 , 74%.

The cyclization precursor was in hand, we examined cyclic acylpalladation trapping by *O*-enolate to obtain 3.⁵⁻⁹ As shown in Table 1, the choice of the base and solvent was very important. We found that AcONa as a base in DMF at 50°C furnished desired 5-*O*-endo cyclized product 3 in 57% yield. Raising reaction temperature and

changing solvent did not improve the yield. We also examined other Pd catalyst such as Pd(PPh₃)₄ and Pd(dba)₂ with ligand such as dppf and dppp. However, the yield of **3** was not improved (Table 1).

Table 1. Cyclic acylpalladation of 4.

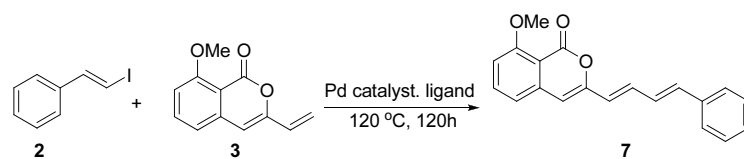


base	solvent	temperature (°C)	yield (%)
Et ₃ N, K ₂ CO ₃ ^{a, 10}	THF	40	35
K ₂ CO ₃ , NH ₂ NH ₂ ¹¹	THF	40	10
Et ₃ N	DMF	50	33
K ₂ CO ₃	DMF	50	16
NaHCO ₃	DMF	50	23
Cs ₂ CO ₃	DMF	50	11
AcONa	DMF	50	57
AcONa	DMF	70	32
AcONa	DMF	100	10
AcONa	toluene	50	14
AcONa	dioxane	50	16
AcONa	DMF	50	33 ^b

^a4.0 eq. of base was used; ^b5 mol% of Pd catalyst was used.

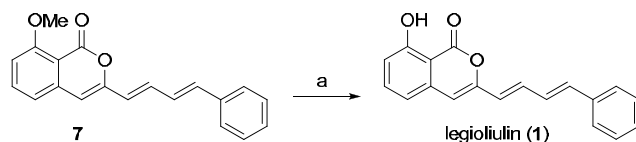
Next, we investigated the construction of the carbon framework of legiolulin (**1**) using Heck reaction. In this reaction, we found that the phosphine ligand greatly affected the reactivity. We previously reported that P(*t*-Bu)₃ was effective ligand to form stilbene framework.¹² Fu reported that the effect of P(*t*-Bu)₃.¹³ According to his report, the effect of P(*t*-Bu)₃ is electron donating as well as accelerating reductive elimination. As a result P(*t*-Bu)₃ afforded desired product **7** in 66% yield (Table 2).

Table 2. Heck reaction of 3 with (1*E*)-1-iodostyrene (2).



catalyst (5 mol%)	base	ligand	solvent	yield (%)
Cl ₂ Pd(PPh ₃) ₂	Et ₃ N	-	DMF	29
Pd(dba) ₂	Cs ₂ CO ₃	PCy ₃	dioxane	24
Pd(dba)₂	Cs₂CO₃	P(<i>t</i>-Bu)₃	dioxane	66

Finally, deprotection of methyl group at C-8 position using BBr_3 in CH_2Cl_2 at -78°C gave legioliulin (**1**). The ^1H and ^{13}C NMR data of the synthetic legioliulin (**1**) were consistent with those of the reported values (Scheme 3).^{2, 14}



Scheme 3. Synthesis of legioliulin (**1**). Reagent and condition: (a) BBr_3 , CH_2Cl_2 , -78°C , 76%.

In conclusion, concise synthesis of legioliulin (**1**) was achieved using acylpalladation and Heck reaction. The studies on biological activities of legioliulin (**1**) is now underway.

Acknowledgements

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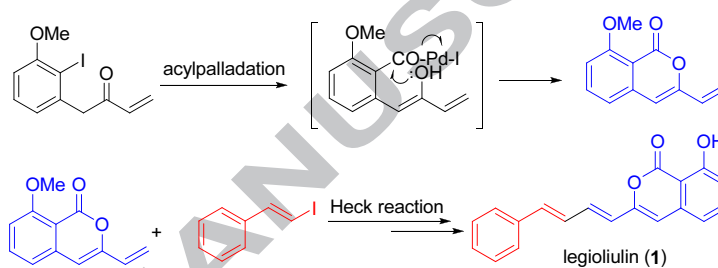
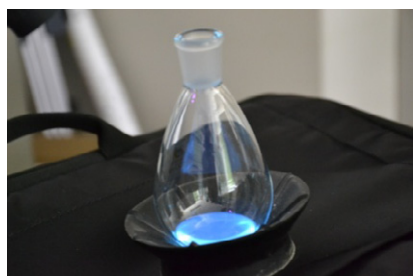
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14. The data of legioliulin (**1**): Mp. 107-108°C; IR (KBr): 3250-3025, 2917, 2849, 1687, 1625, 1609, 1562, 1496, 1460, 1343, 1315, 1223, 1168, 1141, 1090, 1049, 982, 842, 749, 690 cm⁻¹; ¹H NMR (500 MHz, CDCl₃) δ 11.00 (1H, s), 7.55 (1H, t, *J* = 7.5 Hz), 7.45 (2H, d, *J* = 7.5 Hz), 7.34 (2H, t, *J* = 7.5 Hz), 7.26 (1H, t, *J* = 7.5 Hz), 7.22 (1H, dd, *J* = 15.0, 11.0 Hz), 6.92 (1H, d, *J* = 7.5 Hz), 6.89 (1H, dd, *J* = 15.5, 11.0 Hz), 6.86 (1H, d, *J* = 7.5 Hz), 6.81 (1H, d, *J* = 15.5 Hz), 6.39 (1H, s), 6.24 (1H, d, *J* = 15.0 Hz); ¹³C NMR (125 MHz, CDCl₃) δ 166.00, 161.71, 152.06, 137.94, 137.32, 137.03, 136.63, 133.81, 128.80, 128.42, 127.45, 126.83, 122.36, 116.31, 115.24, 106.39, 106.05 ppm. HRMS-EI: *m/z* [M]⁺: calcd for C₁₉H₁₄O₃; 290.0943, found: 290.0945.

Graphical Abstract

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Highlights

First synthesis of legioliulin was accomplished.

Isocoumarin ring of legioliulin was constructed using cyclic acylpalladation.

Chain elongation was performed using Heck reaction using *t*-butylphosphine as a ligand.

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