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

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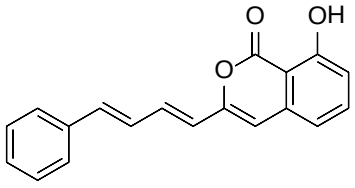
Abstract: Concise synthesis of legioliulin, an isocoumarin compound isolated from *Legionella dumoffii*, was achieved. Isocoumarin ring of legioliulin was constructed using cyclic acylpalladation. Chain elongation was performed using Heck reaction using *t*-butylphosphine as a ligand.

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

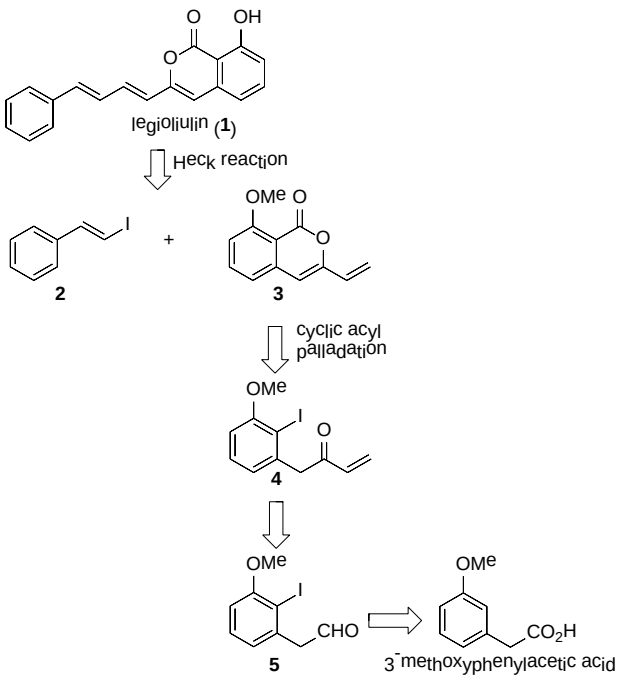
Legionellae, which are responsible for Legionnaire's disease, are facultative intracellular gram-negative bacteria.[1] 10 species of Legionella exhibit blue-white and dark-red autofluorescence. In 2004, Amemura-Maekawa and co-workers isolated legioliulin (**1**) from *Legionella dumoffii*.[2] Legioliulin (**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 consice synthesis of legioliulin (**1**) using cyclic acylpalladation and Heck reaction as the key steps (Figure 1).



legioliulin (**1**)

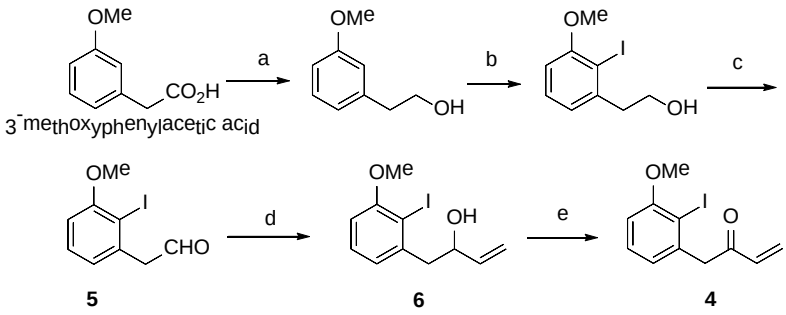
Figure 1. The structure of legioliulin (**1**).

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**).

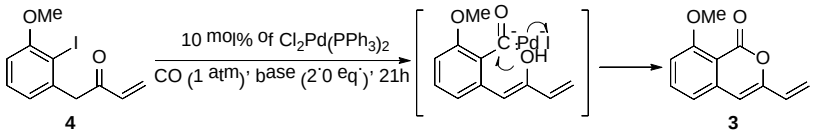
Scheme 2 shows the preparation of cyclization precursor **4**. Reduction of carboxylic acid of 3-methoxyphenylacetic acid with $LiAlH_4$ afforded corresponding alcohol subsequent iodination at *ortho* position and oxidation of primary alcohol gave aldehyde **5**. The yield of iodination was rather low 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*-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 50ºC 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 $Pd(PPh_3)_4$ 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.



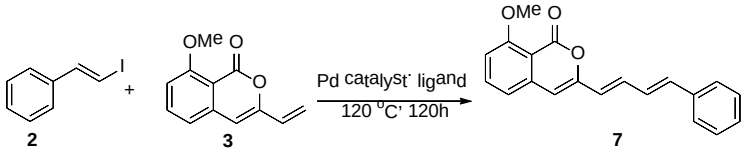
4 3

base	solvent	temperature (°C)	yield (%)
Et_3N, K_2CO_3 [a, 10]	THF	40	35
K_2CO_3, NH_2NH_2[11]	THF	40	10
Et_3N	DMF	50	33
K_2CO_3	DMF	50	16
$NaHCO_3$	DMF	50	23
Cs_2CO_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]

[a] 4.0 eq. of base was used; [b] 5 mol% of Pd catalyst was used.

Next, we investigated the construction of the carbon framework of legioliulin (**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).

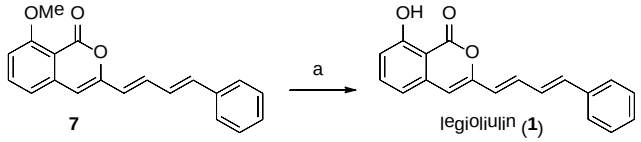
Table 2. Heck reaction of 3 with (1*E*)-1-iodostyrene (2).



2 3 7

catalyst (5 mol%)	base	ligand	solvent	yield (%)
$Cl_2Pd(PPh_3)_2$	Et_3N	-	DMF	29
$Pd(dba)_2$	Cs_2CO_3	PCy_3	dioxane	24
$Pd(dba)_2$	**Cs_2CO_3**	**$P(t\text{-}Bu)_3$**	**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]



7 legioliulin (**1**)

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

This work was supported by JSPS KAKENHI Grant Number 15K07408 to H. M.

References and notes

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14. The data of legioliulin (**1**): Mp. 107-108^{o}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}; ^{1}H NMR (500 MHz, $CDCl_3$) 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); ^{13}C NMR (125 MHz, $CDCl_3$) 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 $C_{19}H_{14}O_3$; 290.0943, found: 290.0945.