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Total synthesis of *ent*-cassa-12,15-diene, a putative precursor of rice phytoalexins, phytocassanes A–E

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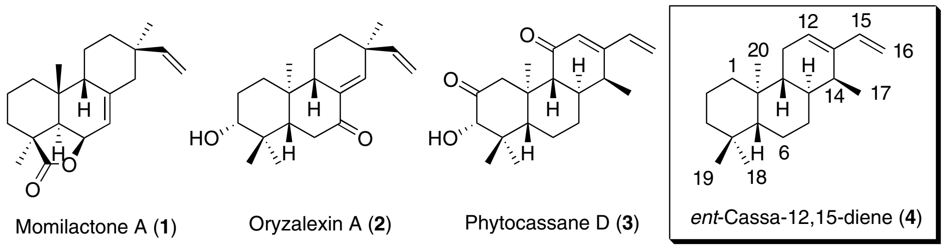
Abstract—The first total synthesis of *ent*-cassa-12,15-diene, a putative precursor of diterpene phytoalexins, was accomplished.

Phytoalexins are antimicrobial secondary metabolites that are biosynthesized de novo and accumulated in plants after exposure to pathogens.[1] Momilactones[2–4] (**1**) and oryzalexins[5,6] (**2**) with a pimarane skeleton and phytocassanes[7,8] (**3**) with a cassane skeleton are polycyclic diterpene phytoalexins isolated from the rice plant infected with a pathogenic microorganism such as *Magnaporthe grisea* (Fig. 1). The absolute structures of oryzalexins and phytocassanes were confirmed by total syntheses.[9,10] However, information about their biosynthetic pathway is limited.[11,12]

Very recently, during the course of functional analysis of a cDNA encoding a diterpene cyclase, OsDTC1, involved in the phytoalexin production in rice, an unknown diterpene hydrocarbon-like compound was found as a major product in the reaction of *ent*-copalyl diphosphate (*ent*-CDP) with the recombinant OsDTC1 expressed in *Escherichia coli* (Scheme 1).[13] The unknown hydrocarbon-like compound was also found in the suspension-cultured rice cells exogenously treated with a chitin elicitor. Since *ent*-CDP is a possible precursor of *ent*-cassa-12,15-diene (**4**), a hypothetical biosynthetic intermediate leading to (−)-phytocassanes recently identified as novel phytoalexins in rice, *ent*-cassa-12,15-diene (**4**), was proposed to be a candidate for the unknown compound. However, only limited structural information was available because of the difficulty of purification of the compound.

In connection with our synthetic studies on phytoalexins,[10] we were interested in the synthesis of *ent*-cassa-12,15-diene (**4**) to clarify the presence of this biologically important compound in rice plant. This paper describes



Momilactone A (**1**) Oryzalexin A (**2**) Phytocassane D (**3**) *ent*-Cassa-12,15-diene (**4**)

Figure 1. Structures of diterpene phytoalexins from rice plant and *ent*-cassa-12,15-diene.

Keywords: synthesis; terpenes and terpenoids; biosynthesis; phytoalexins; dienes.

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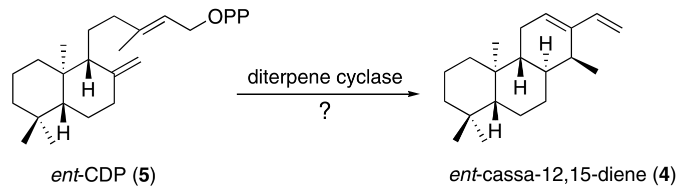
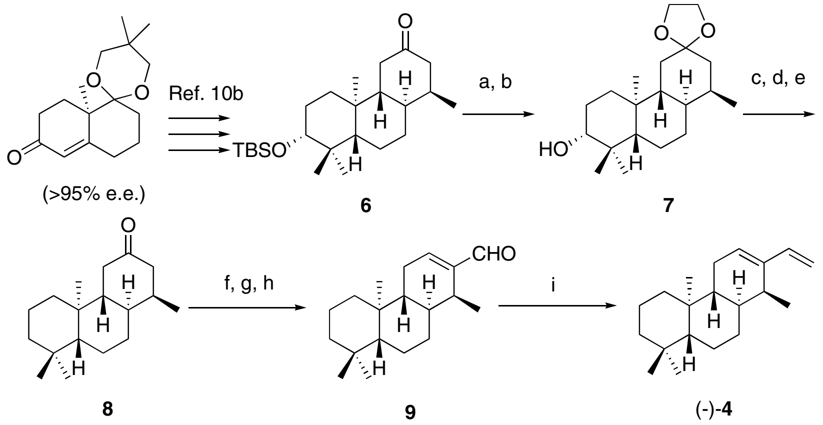


Scheme 1. Enzymatic cyclization of *ent*-CDP.



Scheme 2. Synthesis of (−)-**4**. Reagents and conditions: (a) ethylene glycol, *p*-TsOH, benzene, reflux; (b) TBAF, THF, 60 °C (98% in two steps); (c) TPAP, NMO, MS 4A, CH_3CN–CH_2Cl_2, (quant.); (d) NH_2NH_2, tri(ethylene glycol), 125 °C, 2 h then KOH, 190 °C (86% from **7**); (e) HCl aq, THF (97%); (f) HCO_2Et, NaH, MeOH, −15 °C to rt; (g) ethyl vinyl ether, cat. H_3PO_4; (h) $NaBH_4$, EtOH–THF, 0 °C to rt then HCl aq (52% from **8**); (i) $Ph_3P{=}CH_2$, THF, −78 °C (98%).

the first total synthesis of *ent*-cassa-12,15-diene (**4**), a putative precursor of phytocassanes.

Scheme 2 summarizes our synthesis of *ent*-cassa-12,15-diene (**4**). The known ketone **6**, the intermediate of the total synthesis of phytocassane D,[10b] was selected as the starting material. Protection of the carbonyl group of **6** as acetal was followed by removal of the *tert*-butyldimethylsilyl (TBS) protecting group to give alcohol **7** (98%).[14] For the removal of the oxygen functionality at C-1,[15] the hydroxy group of **7** was first oxidized with tetrapropylammonium perruthenate (TPAP) in the presence of *N*-methylmorpholine *N*-oxide (NMO).[16] Subsequent modified Wolff–Kishner reduction reported by Nagata and Itazaki[17] of the resulting ketone (86%, two steps) was followed by hydrolysis of the acetal to furnish ketone **8** (97%).[18] Regioselective formylation of **8** was followed by protection of the resulting enol with an ethoxyethyl group and reduction of the ketone with $NaBH_4$ then acidic work-up, yielding unsaturated aldehyde **9** (52%, three steps).[18] Finally, Wittig olefination with **9** afforded *ent*-cassa-12,15-diene (**4**) in 98% yield; $[\alpha]_D^{25}$ −58.9 ($c = 0.67$, pentane). The overall yield was 42% from the known ketone **6** in nine steps. All spectroscopic data (IR, ^1H and ^{13}C NMR, EIMS, HRMS) of synthetic (−)-**4** are in perfect accordance with the expected structure of **4**.[19]

The MS spectrum and the retention time of GC analysis of the synthetic *ent*-cassa-12,15-diene (**4**) were identical with those of the sample obtained from the enzymatic reaction of *ent*-CDP (**5**).[13] The unknown hydrocarbon-like compound from the suspension-cultured rice cells and the cyclized product of *ent*-CDP (**5**) catalyzed by the diterpene cyclase is, therefore, *ent*-cassa-12,15-diene (**4**).

In conclusion, the first total synthesis of *ent*-cassa-12,15-diene (**4**) was accomplished in nine steps starting from the known ketone **6**. Since phytocassanes possess an *ent*-cassane skeleton as shown in Figure 1,[10] *ent*-cassa-12, 15-diene (**4**) must be the hypothetical biosynthetic intermediate of phytocassanes. The presence of the biologically important compound in rice plant was clarified by this study.

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19. Properties of synthetic (−)-**4**: colorless oil; $[\alpha]_D^{25}$ −58.9 (*c* 0.67, pentane); GC column: DB-WAX (0.25 mm in diameter, 60 m long, 0.25 μm film thickness), carrier gas He (1 mL/min), 80 °C (2 min) to 250 °C (5 °C/min), $R_t = 36.09$; EIMS (*m/z*) 272 (M^+, 70), 257 (100), 243 (9), 229 (4), 203 (9), 187 (13), 177 (23), 161 (14), 149 (27), 134 (30), 119 (74), 105 (34), 91 (42), 79 (41), 69 (26), 55 (24), and 41 (24); HRMS obsd 272.2495, calcd for $C_{20}H_{32}$ 272.2504; IR ν_{max} (film): 2900 (s, C–H), 1620, 1600 (w, C=C–C=C) cm^{-1}; ^{1}H NMR (400 MHz, CDCl$_3$): $\delta = 0.82$ (s, 3H, 20-CH$_3$), 0.85 (s, 3H, 18-CH$_3$), 0.85–0.97 (m, 1H, 5-H), 0.87 (s, 3H, 19-CH$_3$), 0.92 (d, $J = 7.2$ Hz, 3H, 17-CH$_3$), 1.15–1.67 (m, 12H, 1,2,3,6,7-CH$_2$, 8,9-H), 1.92 (ddd, $J = 4.0$, 9.2, 19.5 Hz, 1H, 11-C*H*H), 2.09 (ddd, $J = 4.0$, 7.2, 19.5 Hz, 1H, 11-CH*H*), 2.38 (dq, $J = 4.4$, 7.2 Hz, 1H, 14-H), 4.89 (d, $J = 11.0$ Hz, 1H, 16-C*H*H), 5.07 (d, $J = 17.5$ Hz, 1H, 16-CH*H*), 5.61 (dd, $J = 4.0$, 4.0 Hz, 1H, 12-H), 6.22 (dd, $J = 11.0$, 17.5 Hz, 1H, 15-H); ^{13}C NMR (100 MHz, CDCl$_3$) $\delta = 13.9$, 14.5, 18.7, 21.5, 22.1, 25.3, 31.0, 32.0, 33.1, 33.6, 35.1, 37.0, 39.3, 42.1, 44.1, 55.3, 109.4, 128.7, 138.7, 141.7.