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Synthesis and biological activity of a stereoisomeric mixture of the mating hormone of *Phytophthora*

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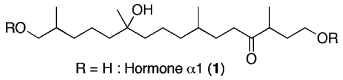
Abstract—The first synthesis of a stereoisomeric mixture of hormone α1, the mating hormone of *Phytophthora*, was achieved, and the synthetic mixture was confirmed to be hormonally active.

Phytophthora, whose name translates as 'plant-destroyer', is one of the most destructive pathogens in the world. In the mid-1840s, late blight, the plant disease caused by a member of this fungus-like genus, destroyed potato crops in Europe and the United States and caused the Irish potato famine.[1] This pathogen, which devastates potato and tomato crops, is known as *Phytophthora infestans*. Another species, *Phytophthora ramorum*, is an aggressive pathogen which is the cause of extensive mortality among oak trees in California;[2] the disease is known as sudden oak death. In Europe, this disease has not been detected in oaks, but has been found to cause twig blight in other plants.[3] In the 20th century, these diseases were managed by effective use of fungicides. However, certain virulent and fungicide-resistant strains have recently undergone extensive migration, causing a worldwide resurgence of late blight in potatoes.[1]

The life cycle of *Phytophthora* species, which are sometimes called water molds as their development is favored by wet conditions, includes characteristic biological events, including sexual reproduction.[1] Each individual is bisexual, capable of producing both female (oogonia) and male (antheridia). There are two mating types, A1 and A2, with sexual reproduction requiring the interaction of both. After sexual reproduction, the oogonia develop into sexual spores called oospores, which can survive harsh conditions such as drying or freezing for months or years in the absence of a living host plant. However, during its asexual phase, *Phytophthora* is an obligate parasite and requires the presence of a living host tissue. In 1929, Ashby proposed that sexual reproduction in *Phytophthora* was regulated by a hormone-like compound,[4] and data were later reported by Galloway and Kouyears supporting a mechanism of chemical stimulation for oospore formation.[5] A factor secreted by the A1 mating type induces the formation of oospores in the A2 mating type, while a factor secreted by A2 induces the formation of oospores in A1. These factors are known as hormones α1 and α2, respectively. Although extensive studies have been conducted with the aim of isolating these hormones, their structures are still obscure due to their scarcity.[6] Recently, Ojika and co-workers finally succeeded in isolating hormone α1 from 1830 L of culture broth of the A1 mating type of another species, *Phytophthora nicotianae*.[7] The structure of hormone α1 was determined as **1** (Fig. 1), a novel diterpene with four stereogenic centers, by extensive MS



R = H : Hormone α1 (**1**)
R = *p*-bromobenzoyl (**2**)

Figure 1. Structures of hormone α1 (**1**) and its bis-*p*-bromobenzoate derivative **2**.

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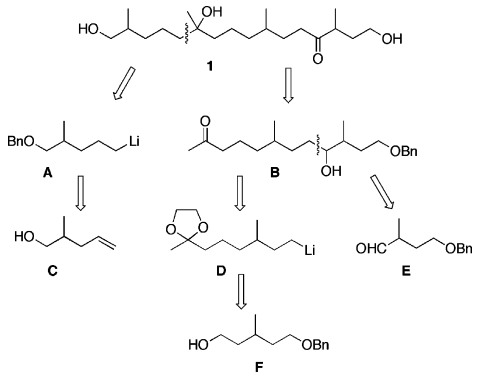
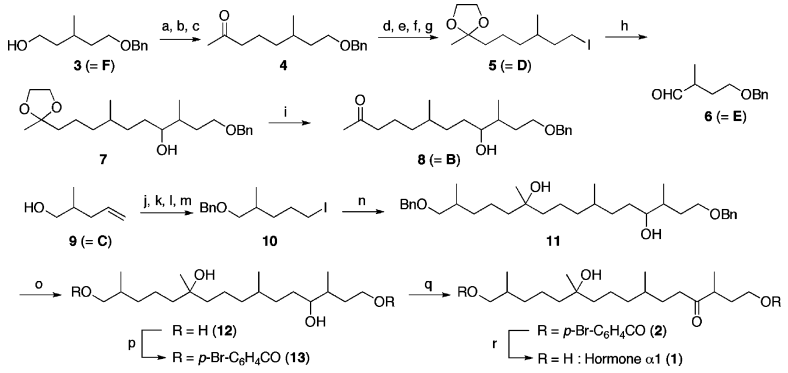
and NMR studies of the natural product and its bis-*p*-bromobenzoate derivative. No information about the relative or absolute configuration of natural **1** was reported. Surprisingly, hormone α1 was found to induce oospore formation not only in *P. nicotianae* but also in *P. capsici*, *P. cambivora*, and *P. infestans*. These results indicate that hormone α1 (**1**) is a universal mating hormone in the heterothallic species of *Phytophthora*. We became interested in synthesizing hormone α1 (**1**) in order to confirm its structure and to investigate its biological activity. This Letter describes the synthesis and biological activity of a stereoisomeric mixture of **1**.

We selected a stereoisomeric mixture of **1** as a target for synthesis. Since diastereomers often give different NMR spectra, it was thought likely that information about the relative configuration of **1** could be obtained by NMR study of the stereoisomeric mixture. Scheme 1 shows a retrosynthetic analysis of **1**. Hormone α1 (**1**) would be synthesized from fragments **A** and **B** via nucleophilic addition. Fragment **B** would in turn be derived from nucleophilic addition of fragment **D** to the known aldehyde **E**. The known and easily prepared compounds **C**, **E**, and **F** were selected as starting materials. Scheme 2 summarizes our synthesis of **1**. First, fragment **D** was prepared from the known alcohol **3**[8] (=**F**). Tosylation of **3** yielded a tosylate which gave the corresponding iodide on treatment with NaI (73% in two steps),[9] and alkylation of ethyl acetoacetate with the resulting iodide furnished a β-ketoester derivative which was saponified to give ketone **4** (67% in two steps). After protection of the carbonyl group (95%), the benzyl ether was removed by hydrogenolysis (98%), and the resulting alcohol was converted to iodide **5** by tosylation followed by treatment with NaI (77% in two steps). Halogen–metal exchange of **5** with *t*-BuLi followed by coupling with known aldehyde **6**[10] (=**E**) afforded **7** in 90% yield. Acid hydrolysis of the ketal of **7** gave ketone **8** (=**B**). The precursor (**10**) of fragment **A** was derived from known alcohol **9**[11] (=**C**) in four steps: protection of the hydroxyl group as a benzyl ether (77%), hydroboration–oxidation (54%), tosylation (84%) and treatment with NaI (99%). The iodide **10** was lithiated with *t*-BuLi, and the resulting organolithium was coupled with ketone **8** to afford **11** in 78% yield. Deprotection of the two benzyl groups by hydrogenolysis gave tetraol **12** (91%). As the *p*-bromobenzoyl derivative (**2**) of the natural hormone α1 (**1**) has already been prepared by Ojika et al. and its physical properties reported,[7] a *p*-bromobenzoyl group was selected for protection of the two primary hydroxyl groups (60%). Oxidation of the remaining secondary hydroxyl group with Dess–Martin periodinane gave **2**[12] (83%), and hydrolysis of the *p*-bromobenzoyl groups gave a stereoisomeric mixture of **1** in 83% yield.[13] The overall yield of **1**, based on **3**, was 9.2% after 15 steps. The ^{1}H and ^{13}C NMR spectra of synthetic **1** and **2** were in good agreement with the previously reported data.[7] The structure of the natural hormone α1 (**1**) was confirmed by these results. Unfortunately, both of the synthetic samples gave simple spectra on ^{1}H and ^{13}C NMR analyses, as though they contained only a single diastereomer. Therefore, no information about the relative stereochemistry of **1** was obtained; in other words, it was impossible to discriminate between the diastereomers of **1** and **2** by NMR analysis.



Scheme 1. Retrosynthetic analysis of hormone α1 (**1**).

The oospore-inducing activity of a sample of synthetic **1** was evaluated according to the previously reported procedure.[7] Figure 2 shows the results of oospore induction,



Scheme 2. Synthesis of hormone α1 (**1**). Reagents, conditions and yields: (a) (i) *p*-TsCl, py, (ii) NaI, acetone (73%); (b) ethyl acetoacetate, K_2CO_3, acetone, reflux (74%); (c) KOH aq, EtOH reflux, then HCl aq (90%); (d) $HO(CH_2)_2OH$, PTSA, benzene, reflux (95%); (e) H_2, Pd/C, MeOH (98%); (f) *p*-TsCl, py (77%); (g) NaI, $NaHCO_3$, acetone (quant.); (h) *t*-BuLi, ether, −78°C, then **6** (90% based on **6**, 65% based on **5**); (i) PTSA, EtOH, H_2O (quant.); (j) NaH, BnBr, THF (77%); (k) $NaBH_4$, BF_3OEt_2, THF, then H_2O_2 aq (54%); (l) *p*-TsCl, py (84%); (m) NaI, acetone (99%); (n) *t*-BuLi, ether, −78°C, then **8** (78% based on **8**); (o) H_2, Pd/C, MeOH (91%); (p) *p*-Br-C_6H_4COCl, py (60%); (q) Dess–Martin periodinane, CH_2Cl_2 (83%); (r) K_2CO_3, MeOH (83%).

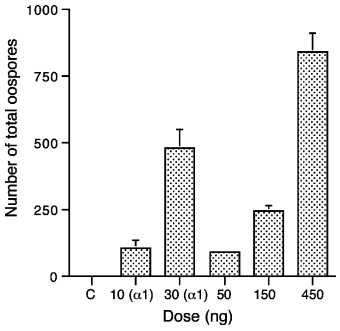


Figure 2. The dose-dependent increase of oospore formation in the A2 mating type of *P. nicotianae* induced by synthetic sample (ng/disc) in comparison with natural hormone α1 (α1) at doses of 10 and 30 ng/disc. Values are means of four replicates. C: control.

with doses of 50–450 ng/disk, for the A2 mating type of *P. nicotianae*. The values for the natural product were recorded at 10 and 30 ng/disk. The synthetic stereoisomeric mixture of **1** did exhibit oospore-inducing activity, but its activity was estimated to be about five times weaker than that of the natural product. This result indicates that some stereoisomers of **1** show hormonal activity, but the other isomers do not inhibit activity.

In summary, we achieved the first synthesis of a stereoisomeric mixture of hormone α1 (**1**). The synthetic mixture was found to induce the formation of oospores. Since the activity of synthetic **1** was about five times weaker than that of the natural product, it was concluded that some stereoisomers of **1** show hormonal activity. The development of a stereoselective synthesis of **1** and investigation of the relationship between stereochemistry and biological activity are currently under way.

Acknowledgment

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12. Properties of synthetic **2**: colorless oil; IR (KBr) ν_{max} (cm^{-1}) = 3545 (br s, –OH), 2940 (br s), 2355 (w), 1715 (s, C=O), 1590 (s), 1485 (m), 1465 (m), 1395 (s), 1270 (s), 1175 (m), 1115 (s), 1070 (m), 1010 (m), 965 (w), 850 (m), 755 (s), 710 (w), 685 (m), 630 (w), 475 (w); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 0.84$ (m, 3H, 19-CH_3), 1.03 (d, $J = 6.8$ Hz, 3H, 17-CH_3), 1.10–1.60 (m, 15H), 1.16 (s, 3H, 18-CH_3), 1.17 (d, $J = 6.8$ Hz, 3H, 20-CH_3), 1.77 (m, 1H, 2-C*H*H), 1.94 (m, 1H, 15-CH), 2.20 (m, 1H, 2-CH*H*), 2.36–2.57 (m, 2H, 5-CH_2), 2.73 (sxt, $J = 6.8$ Hz, 1H, 3-H), 4.11 (dd, $J = 6.0$, 10.7 Hz, 1H, 16-C*H*H), 4.21 (dd, $J = 6.8$, 10.7 Hz, 1H, 16-CH*H*), 4.31 (br t, $J = 6.3$ Hz, 2H, 1-CH_2), 7.57–7.92 (m, 8H, Ar-H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 16.9$, 19.18, 19.21, 21.1, 26.7, 30.4, 31.3, 32.2, 32.3, 32.6, 33.9, 37.2, 37.3, 39.0, 41.9, 42.0, 43.01, 43.04, 63.2, 69.9, 72.5, 127.8, 128.0, 128.9, 129.2, 131.0, 131.56, 131.61, 165.6, 165.8, 213.7; HRMS (FAB): calcd. for $C_{34}H_{46}{}^{79}Br_2O_6Na$ 731.1559, found 731.1556 $[M+Na]^+$. ^{1}H and ^{13}C NMR spectra are in good accordance with those of the reported natural product.
13. Properties of synthetic **1**: colorless oil; IR (KBr) ν_{max} (cm^{-1}) = 3395 (br s, –OH), 2935 (br s), 2350 (w), 1705 (s, C=O), 1465 (w), 1370 (w), 1055 (m), 565 (br m); ^{1}H NMR (400 MHz, CD_3OD): $\delta = 0.80$ (d, $J = 6.3$ Hz, 3H, 19-CH_3), 0.82 (d, $J = 6.8$ Hz, 3H, 17-CH_3), 0.98 (d, $J = 6.8$ Hz, 3H, 20-CH_3), 0.99 (m, 1H, 14-CH*H*) 1.04 (s, 3H, 18-CH_3), 1.05 (m, 1H 8-CH*H*), 1.15–1.56 (m, 15H), 1.80 (m, 1H, 2-CH*H*), 2.45 (m, 2H, 5-CH_2), 2.68 (sxt, $J = 6.8$ Hz, 1H, 3-CH), 3.22 (dd, $J = 6.8$, 10.7 Hz, 1H, 16-CH*H*), 3.32 (dd, $J = 5.9$, 10.7 Hz, 1H, 16-CH*H*), 3.43 (br t, $J = 6.8$ Hz, 2H, 1-CH_2); ^{13}C NMR (100 MHz, CD_3OD): $\delta = 16.9$ (C-20), 17.1 (C-17), 19.9 (C-19), 22.3 (C-9,13), 26.9 (C-18), 31.7 (C-6), 33.6 (C-7), 35.0 (C-14), 36.7 (C-2), 36.9 (C-15), 38.6 (C-8), 40.0 (C-5), 43.0 (C-10,12), 44.0 (C-3), 60.6 (C-1), 68.4 (C-16), 73.4 (C-11), 217.5 (C-4); HRMS (FAB): calcd. for $C_{20}H_{40}O_4Na$ 367.2825, found 367.2823 $[M+Na]^+$. ^{1}H and ^{13}C NMR spectra are in good accordance with those of the reported natural product.