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Stereochemical analysis of α1, a mating hormone of the phytopathogen *Phytophthora*

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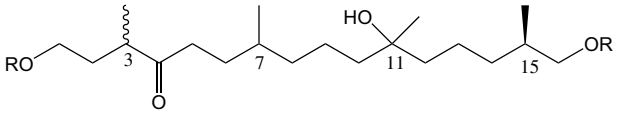
Abstract—The stereochemistry of α1, the first identified mating hormone of the plant pathogen *Phytophthora*, has been unknown due to its acyclic flexible nature. Here, two stereogenic centers of α1 are determined to be (3*RS*,15*R*)-configuration by NMR analysis of the Mosher's esters of α1 and a synthetic model compound. The information obtained here will be helpful for reducing the burden of the researchers who are trying to synthesize all the possible stereoisomers of α1 to elucidate its full stereochemistry.

1. Introduction

The fungus-like microorganisms of the genus *Phytophthora* include notorious plant pathogens that can devastate important agricultural products, such as potatoes and tomatoes. The heterothallic members of *Phytophthora* utilize mating hormones for their sexual reproduction, resulting in the formation of sexual spores known as oospores,[1,2] which greatly contribute to their durability and pathogenic success.[3] In 2005, one of the mating hormones, α1 **1**, was identified and characterized from the A1 mating type of *Phytophthora nicotianae*.[4] This substance induced oospores in the counter mating type A2 at a nanogram level. The mating hormone α1 **1** is an acyclic oxygenated diterpene, with four discrete stereogenic centers. Although the determination of the absolute configuration is essential for further chemical biological researches, the flexibility of the molecular skeleton, as well as the unavailability of **1** (1.2 mg from ca. 1800 L culture broth),[4] hampers the stereochemical analysis. Recently, an isomeric mixture that could contain all possible stereoisomers of **1** has been synthesized and its hormonal activity demonstrated,[5] suggesting that the synthetic mixture contains **1**. However, the stereoselective total synthesis of the 16 possible stereoisomers will be needed without any stereochemical information on the natural specimen of **1**. To aid the future total synthesis of **1**, we examined the absolute configuration of the two terminal stereogenic centers, C-3 and C-15, by the NMR analysis of the Mosher's esters of **1**.



1: R = H
(*R*)-**2**: R = (*R*)-MTPA
(*S*)-**2**: R = (*S*)-MTPA

2. Results and discussion

A hundred micrograms of the mating hormone α1 **1** were first converted to bis-[α-methoxy-α-(trifluoromethyl)phenylacetate] (bis-MTPA) esters, (*R*)-**2** and (*S*)-**2**.[6] The proton chemical shifts of the oxymethylene groups (H-1 and H-16) were affected by the neighboring MTPA esters, providing us with valuable information about their stereochemistry. The ^{1}H NMR spectra indicated that both (*R*)-**2** and (*S*)-**2** were mixtures of stereoisomers, suggesting that α1 **1** was originally an epimeric mixture. This could be supported by the facts that the epimers ratio was not particularly affected by the esterification time (data not shown) and that the synthetic model ketone **3** was converted to the corresponding MTPA esters without epimerization (vide infra). The major isomer of (*R*)-MTPA ester (*R*)-**2** (60:40 ratio) indicated splitting methylene signals both for H-1 (δ 4.23 and 4.37, $\Delta\delta = 0.14$) and for H-16 (δ 4.08 and 4.25, $\Delta\delta = 0.17$) (Fig. 1a). On the other hand, the major isomer of (*S*)-MTPA ester (*S*)-**2** (64:36 ratio) indicated overlapping methylene signals both for H-1 (δ 4.34, $\Delta\delta = 0$) and

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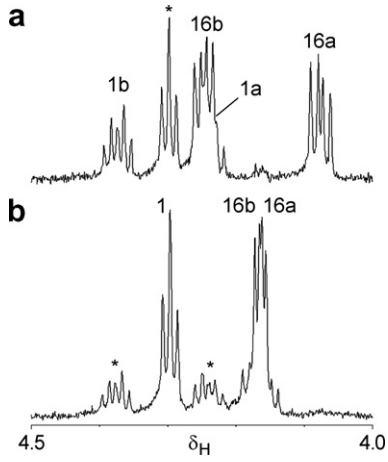


Figure 1. Partial ^{1}H NMR spectra of (*R*)-**2** (a) and (*S*)-**2** (b) in $CDCl_3$. The signals due to the minor epimers [40% in (*R*)-**2**, 36% in (*S*)-**2**] are indicated with asterisks.

Table 1. Chemical shift differences of two oxymethylene protons of MTPA esters

MTPA esters	Δδ (*R*-MTPA)	Δδ (*S*-MTPA)
(*R*)-**2**, (*S*)-**2** (H-16)	0.17	0.03
(*S*)-RCH(CH_3)C**H**$_2$OMTPA[6]	0.00–0.05	0.14–0.20
(*R*)-**2**, (*S*)-**2** (H-1)	0.14	0.00
(*R*)-**7**, (*S*)-**7**	0.00	0.14

for H-16 (δ 4.15 and 4.18, $\Delta\delta = 0.03$) (Fig. 1b). Kobayashi et al. studied the absolute configuration of the primary alcohols with a β-methyl branch [R–CH(CH_3)–CH_2OH] and reported that the (*R*)- and (*S*)-MTPA esters of (*S*)-alcohols indicated overlapping ($\Delta\delta = 0$–0.05) and splitting ($\Delta\delta = 0.14$–0.20) methylene signals, respectively, (Table 1).[7] Since the opposite phenomenon was observed for the oxymethylene protons (H-16) of (*R*)-**2** ($\Delta\delta = 0.17$) and (*S*)-**2** ($\Delta\delta = 0.03$) (Table 1), the (15*R*)-configuration of α1 **1** was determined.

To determine the absolute configuration of C-3, the NMR data for the MTPA esters of γ-methyl-branched primary alcohols [R–CH(CH_3)–CH_2CH_2OH] are needed. Although some examples of simple alcohols (R = alkyl)[8,9] and a δ-hydroxy compound (R = R′CH(OH)–)[10] are already known, the absolute configuration of δ-oxo compounds (R = R′CO–), such as α1 **1** has not been examined. Therefore, a model compound that possesses the same substructure (C-1 to C-6) as that of **1**, (*S*)-1-hydroxy-3-methyl-4-octanone **3**, was synthesized (Scheme 1). (*S*)-2-Methyl-1,4-butandiol was converted to monosilyl ether **4**[11] with 3:1 regioselectivity. After chromatographic separation, **4** was oxidized to aldehyde **5**.[11] Addition reaction with buthyllithium followed by oxidation gave ketone **6**.[12] Deprotection of the silyl group furnished the desired keto alcohol **3**.[13] Esterification of **3** with MTPA chlorides gave (*R*)- and (*S*)-MTPA esters, (*R*)-**7** and (*S*)-**7**, respectively.

The ^{1}H NMR of (*R*)-**7** and (*S*)-**7** indicated overlapping ($\Delta\delta = 0$) and splitting ($\Delta\delta = 0.14$) oxymethylene signals, respectively, (Table 1, Fig. 2). Since the opposite phenomenon was observed for the oxymethylene protons (H-1) of the major isomers of (*R*)-**2** ($\Delta\delta = 0.14$) and (*S*)-**2** ($\Delta\delta = 0$) (Table 1, Fig. 1), the (3*R*)-configuration of the major epimer of α1 **1** was also determined.

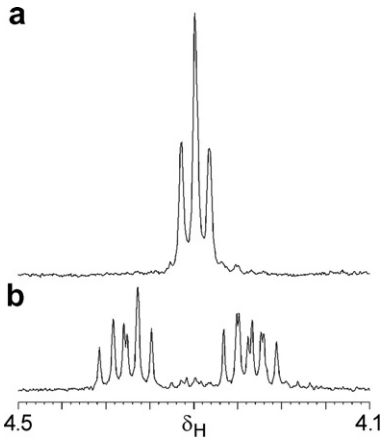
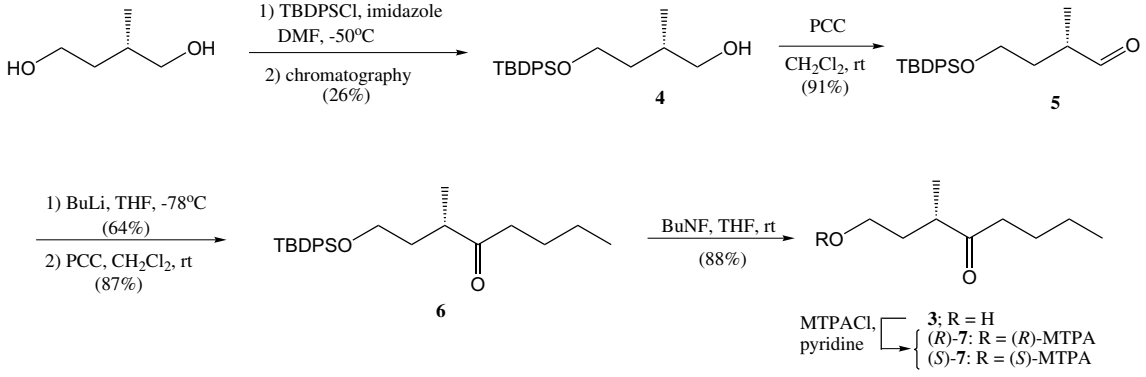


Figure 2. ^{1}H NMR signals due to the oxymethylene protons of (*R*)-**7** (a) and (*S*)-**7** (b) in $CDCl_3$.

3. Conclusion

The absolute configuration of the *Phytophthora* mating hormone α1 **1** was partially determined as 3*RS*,15*R* (3*R*/3*S* = ca. 3:2) by NMR analysis of the Mosher's esters of



Scheme 1. Synthesis of model compound **3** and its MTPA esters (*R*)-**7** and (*S*)-**7**.

1 and the synthetic model compound **3**. This stereochemical outcome has limited the number of the possible stereoisomers of **1** from sixteen to four. The total synthesis of the four stereoisomers, as well as the evaluation of their hormonal activity, will be essential for determining the full stereochemistry of α1 (**1**).

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6. Compound α1 **1** (50 μg) was treated with (*S*)-MTPA chloride (6 μL) in dry pyridine (0.2 mL) for 19 h. The crude product was purified by HPLC [Develosil ODS-UG-5 (ϕ 4.6 × 250 mm), 75–100% MeOH in H_2O in a 60 min linear gradient, flow rate 1 mL min^{-1}, detection at 220 nm] to give (*R*)-MTPA ester (*R*)-**2** (ca. 0.1 mg, t_R = 39.3 min): ^{1}H NMR for the major isomer (600 MHz, $CDCl_3$) δ 7.51 (m, 4H, Ph), 7.41 (m, 6H, Ph), 4.37 (dt, J = 11.4, 6.2 Hz, 1H, H-1b), 4.25 (dd, J = 10.6, 5.8 Hz, 1H, H-16b), 4.24 (m, 1H, H-1a), 4.08 (dd, J = 10.6, 6.6 Hz, 1H, H-16a), 3.55 (s, 3H, OMe), 3.49 (s, 3H, OMe), 2.55 (m, 1H, H-3), 2.46 (m, 1H, H-5b), 2.30 (m, 1H, H-5a), 2.08 (m, 1H, H-2b), 1.87 (m, 1H, H-15), 1.67 (m, 1H, H-2a), 1.58 (m, 1H, 6b), 1.42–1.22 (m, 13H, H-6a, 7, 8b, 9, 10, 12, 13, 14b), 1.17 (m, 1H, H-14a), 1.14 (s, 3H, 11-Me), 1.11 (m, 1H, H-8a), 1.08 (d, J = 7.2 Hz, 3H, 3-Me), 0.92 (d, J = 6.6 Hz, 3H, 15-Me), 0.84 (d, J = 6.6 Hz, 3H, 7-Me). Under the same conditions, but using (*R*)-MTPA chloride, **1** (50 μg) was converted to (*S*)-MTPA ester (*S*)-**2** (ca. 0.1 mg, t_R = 38.5 min): ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.51 (m, 4H, Ph), 7.41 (m, 6H, Ph), 4.30 (t, J = 6.0 Hz, 2H, H-1), 4.18 (dd, J = 10.6, 6.0 Hz, 1H, H-16b), 4.15 (dd, J = 10.6, 5.4 Hz, 1H, H-16a), 3.55 (s, 3H, OMe), 3.53 (s, 3H, OMe), 2.59 (m, 1H, H-3), 2.42 (m, 1H, H-5b), 2.34 (m, 1H, H-5a), 2.08 (m, 1H, H-2b), 1.87 (m, 1H, H-15), 1.67 (m, 1H, H-2a), 1.58 (m, 1H, 6b), 1.42-1.22 (m, 13H, H-6a, 7, 8b, 9, 10, 12, 13, 14b), 1.15 (m, 1H, H-14a), 1.14 (s, 3H, 11-Me), 1.10 (m, 1H, H-8a), 1.09 (d, J = 7.2 Hz, 3H, 3-Me), 0.93 (d, J = 6.6 Hz, 3H, 15-Me), 0.86 (d, J = 6.6 Hz, 3H, 7-Me).
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12. Compound **6**: colorless oil; $[\alpha]_D^{26}$ = +8.0 (*c* 0.13, $CHCl_3$); IR (film) 1713, 1112, 702, 504 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.64 (m, 4H), 7.38 (m, 6H), 3.65 (t, J = 6.0 Hz, 2H), 2.79 (sextet, J = 6.8 Hz, 1H), 2.42 (m, 2H), 1.94 (m, 1H), 1.51 (m, 3H), 1.28 (m, 2H), 1.05 (s, 9H), 1.03 (d, J = 7.2 Hz, 3H), 0.89 (t, J = 7.6 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 214.6, 135.5 (4C), 133.8 (2C), 129.6 (2C), 127.6 (4C), 61.7, 42.5, 41.0, 35.5, 26.9 (3C), 25.8, 22.4, 19.2, 16.3, 13.9. Anal. Calcd for $C_{25}H_{36}O_2Si$: C, 75.75; H, 9.09; N, 0.00. Found: C, 75.67; H, 9.28; N, 0.00.
13. Compound **3**: colorless oil; $[\alpha]_D^{25}$ = −7.7 (*c* 0.25, $CHCl_3$); IR (film) 3421 (br), 1709, 1054 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 3.63 (m, 2H), 2.74 (sextet, J = 7.2 Hz, 1H), 2.48 (m, 2H), 1.94 (m, 1H), 1.65–1.50 (m, 3H), 1.31 (sextet, J = 7.2 Hz, 2H), 1.12 (d, J = 7.2 Hz, 3H), 0.91 (t, J = 7.2 Hz, 3H); ^{13}C NMR δ 215.3, 60.6, 43.2, 40.9, 35.4, 25.8, 22.4, 16.6, 14.0. Anal. Calcd for $C_9H_{18}O_2$: C, 68.35; H, 11.39; N, 0.00. Found: C, 68.35; H, 11.48; N, 0.00.