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Chemical Modification of Pseurotin A: One-pot Synthesis of Synerazol and Pseurotin E and Determination of Absolute Stereochemistry of Pseurotin E

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Abstract Two natural products, synerazol and pseurotin E, were synthesized from the natural product pseurotin A in 58% and 57% yields, respectively in one-pot procedures. This work also establishes the absolute stereochemistry of pseurotin E.

Keywords pseurotin A, synerazol, pseurotin E, chemical modification, absolute stereochemistry

Pseurotin A (**1**) is a microbial secondary metabolite isolated from the fermentation broth of *Pseudeurotium ovalis* STOLK (Ascomycetes) in 1976 [1]. It has various biological activities, including induction of cell differentiation [2], inhibition of chitin synthase [3], inhibition of monoamine oxidase [4], and apomorphine-antagonistic activity [5]. Pseurotin A (**1**) possesses a highly substituted 1-oxa-7-azaspiro[4,4]non-2-ene-4,6-dione skeleton. The structure and absolute stereochemistry of **1** have been determined by a single-crystal X-ray analysis of its 12,13-dibromo derivative [6]. The core structure of **1** is found in other natural products, too (Fig. 1). These pseurotin-related natural products also possess interesting biological activities, including apomorphine-antagonistic activity in the cases of pseurotin D [5] and F [7], anti-angiogenic activity [8a], antifungal activity [8b] and synergistic activity [8b] in the case of synerazol (**2**) isolated

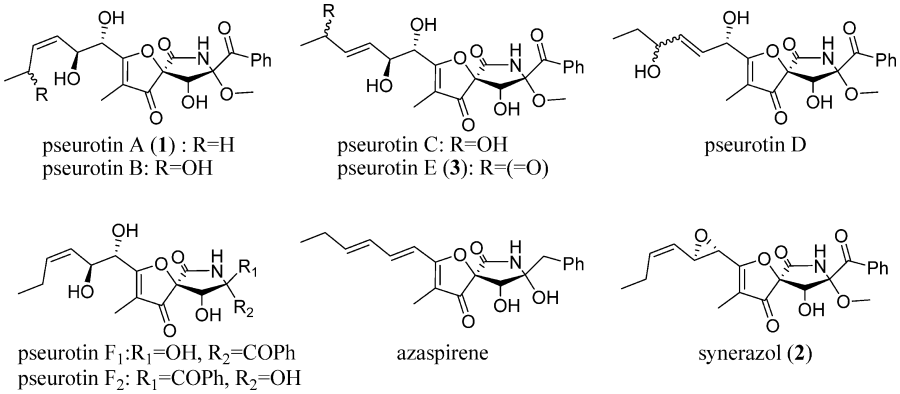

Fig. 1 Structure of pseurotin-related natural products.

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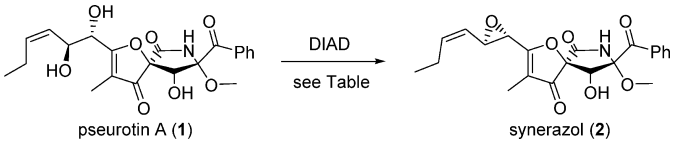
from the fermentation broth of *Aspergillus fumigatus* SANK 10588 in 1991 [8b], and anti-angiogenic activity in the case of azaspirene [9]. Another member of the pseurotin family, pseurotin E (**3**), was isolated from the fermentation broth of *Pseudeurotium ovalis* STOLK in 1981 [10], though its absolute stereochemistry and biological activity were not established.

Elegant total syntheses of **1** [11, 12], pseurotin F_2 [11, 12], **2** [13] and azaspirene [14, 12] have been reported by Hayashi *et al.* and Tadano *et al.* However, these syntheses involved many steps, and are unsuitable for large-scale application to provide samples for biological testing. On the other hand, the supply of pseurotin-related natural products from fermentation is also limited, except for **1**. The reported fermentation yields are 40 mg/liter for **1** [10], 1.4 mg/liter for **2** [8b], and 6 mg/liter for **3** [10]. Hence, we thought chemical modification of **1** might be an attractive way to obtain large amounts of pseurotin-related natural products. In this paper, we wish to report one-pot synthesis of synerazol (**2**) and pseurotin E (**3**) from pseurotin A (**1**) and determination of the absolute stereochemistry of **3**. Various chemical modifications of pseurotin-related natural products, including acetylation (pseurotin A [1] and synerazol [16]), hydrogenation (pseurotin A [15], B [10], C [10]), oxidative cleavage (pseurotin A [1], B [10], C [10]), dibromination [1] (pseurotin A), formation of acetonide [1] (pseurotin A), reduction of carbonyl group [15] (pseurotin A), and synthesis of pseurotin A from synerazol for the purpose of the determination of its absolute stereochemistry [16], have been reported.

Compound **1** was obtained from the fermentation broth of *Aspergillus* sp. The synthesis of **2** from **1** is illustrated in Table 1. First of all, epoxidation of the 1,2-diol of **1** under the reported Mitsunobu condition [17] did not afford **2** (entry 1). However, this reaction proceeded when Cy_3P was replaced with PPh_3 (entry 2). To optimize the reaction, the effects of solvents and temperature were examined. Dichloroethane (DCE) gave a better yield (entry 3). Decreasing the temperature reduced the formation of minor by-products (58% yield, entry 4). Synthetic **2** exhibited properties identical to those of the natural product [8b] (^{1}H-NMR, ^{13}C-NMR, and optical rotation [18]). We were able to obtain **2** on a 200 mg scale.

We decided to synthesize the (10*R*,11*S*)-diastereoisomer of **2** to confirm the diastereoselectivity in the synthesis of **2**. The (10*R*,11*S*)-diastereoisomer **4** is generated if the hydroxyl group at the 10 position is eliminated under the above Mitsunobu conditions. NMR spectra of the (10*R*,11*S*)-diastereoisomer **4** have not been reported, although its total synthesis was achieved [13]. Selective

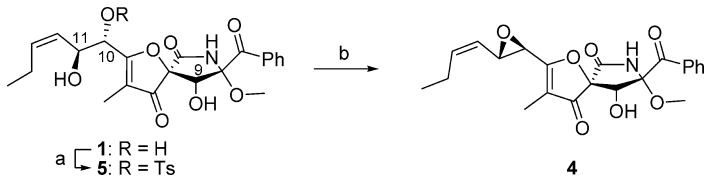
Table 1 Synthesis of synerazol by Mitsunobu reaction



pseurotin A (**1**) → synerazol (**2**)

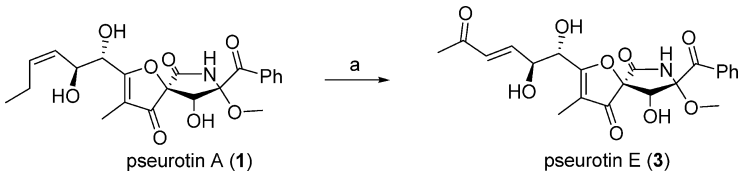
Entry	Phosphine	Solvents	Temp (°C)	Time (hours)	Yield of **2** (%)
1	Cy_3P	THF	22 to 40	25	0
2	Ph_3P	THF	22 to 60	28	14
3	Ph_3P	DCE	30	4.5	43
4	Ph_3P	DCE	15	7	58
5	Ph_3P	DCM	15	3	54

DIAD: diisopropyl azodicarboxylate; Cy: cyclohexyl; DCE: dichloroethane.



Scheme 1 Synthesis of **4**[a].

[a] Reagents: (a) TsCl, Et_3N, *n*-Bu_2SnO, DCM, rt, 17 hours, 33%; (b) K_2CO_3, MeOH, 4°C, 2 hours, 27%.



Scheme 2 Synthesis of pseurotin E[a].

[a] Reagents: (a) Grubbs catalyst (second generation), methyl vinyl ketone, DCM, 40 °C, 2.5 hours, 57%.

tosylation reaction of the 1,2-diol of **1** was achieved in the presence of dibutyltin oxide [19] to give **5** (Scheme 1). Regioselectivity of the tosyl group was assigned on the basis of ^{1}H-NMR multiplicity (H-C11 (ddd, J=5.1, 7.8, 9.5 Hz), H-C10 (d, J=7.8 Hz), and H-C9 (d, J=12.4 Hz)). Epoxidation under basic conditions gave **4**. The optical rotation of **4** ($[\alpha]_D^{26}$ −53.9° (c 0.075, $CHCl_3$)) is in good agreement with literature data [13] ($[\alpha]_D^{22}$ −49.2° (c 0.02, $CHCl_3$)). The (10*R*,11*S*)-diastereomer **4** [20] proved readily distinguishable from **2** by ^{1}H-NMR, ^{13}C-NMR, and HPLC, thereby confirming the complete diastereoselectivity of the transformation leading to **2**.

Next, we envisioned that **3** could be derived from **1** by olefin cross metathesis. Treatment of **1** with methyl vinyl ketone and second-generation Grubbs catalyst (1,3-bis-(2,4,6-trimethylphenyl)-2-imidazolidinylidene)dichloro(*o*-isopropoxyphenylmethylene)ruthenium) [21] afforded **3** in 57% yield (Scheme 2). The ^{1}H-NMR and ^{13}C-NMR spectra of synthetic **3** [22] were in good accordance with those reported for the natural product [10]. Hence, the absolute stereochemistry of pseurotin E (**3**) was defined. This metathesis was also applicable to the synthesis of other pseurotin E analogues (data not shown).

In summary, we have developed an efficient one-pot synthesis of synerazol and pseurotin E from readily available pseurotin A. This work also establishes the absolute stereochemistry of pseurotin E. We will report syntheses of pseurotin A, synerazol and various pseurotin E analogs, as well as their biological activities, in due course.

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20. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 1.03 (3H, t, *J*=7.6 Hz), 1.82 (3H, s), 2.16–2.32 (2H, m), 3.39 (3H, s), 3.73 (1H, d, *J*=1.9 Hz), 4.02 (1H, ddd, *J*=0.7, 2.0, 7.8 Hz), 4.07 (1H, d, *J*=12.7 Hz), 4.63 (1H, d, *J*=12.5 Hz), 5.07 (1H, ddt, *J*=1.5, 9.0, 10.7 Hz), 5.85 (1H, br dt, *J*=11.0, 7.8 Hz), 7.31 (1H, br s), 7.50 (2H, br dd, *J*=7.3, 8.3 Hz), 7.65 (1H, br t, *J*=7.3 Hz), 8.29 (2H, dd, *J*=1.3, 8.3 Hz); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 5.1, 14.0, 21.3, 51.7, 53.1, 55.1, 74.0, 89.4, 91.8, 113.8, 123.6, 128.8, 130.5, 132.4, 134.7, 141.3, 164.9, 182.2, 194.2, 196.8; $[\alpha]_D^{26}$ −53.9° (*c* 0.075, $CHCl_3$), HPLC (Inertsil ODS-2) retention time: 14.9 minutes (synerazol), 15.7 minutes (**4**).
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