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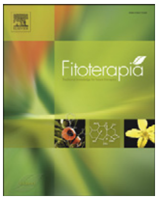

# A convenient and efficient one-pot conversion of peimisine into cyclopamine

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ABSTRACT

A convenient and efficient one-pot synthesis of cyclopamine from peimisine is described. The key steps involve one-pot hydrazination and subsequent Bamford–Stevens reaction. The mild reaction conditions, high overall yield as well as an easy purification indicate this process can potentially be used for the scale-up preparation of cyclopamine.

## 1. Introduction

The hedgehog signaling pathway is essential for embryogenesis and for tissue homeostasis in the adult [1,2]. However, it may induce malignancies in a number of tissues when constitutively activated, and it may also have a role in other forms of normal and maladaptive growth. Cyclopamine is a naturally occurring isosteroidal alkaloid, which specifically inhibits the hedgehog pathway by binding directly to smoothened, an important hedgehog response element [3–6]. To use cyclopamine as a tool to explore and inhibit the hedgehog pathway in vivo, a substantial quantity is required, and as a practical matter cyclopamine has been effectively unavailable for usage in animals larger than mice [7].

The total synthetic approaches to obtain cyclopamine have been reported. First, the synthesis of 17-acetyl-5α-etiojerva-12, 14, 16-trien-3β-ol from simple chemicals has been achieved by Johnson et al. [8]. Masamune et al. also report its conversion into the cyclopamine relative jervine [9]. More recently, the chemical synthesis from known steroid to cyclopamine has been achieved successfully [10]. In addition, the Wolff-Kishner reduction of jervine has been reported to yield cyclopamine [11–13] (Scheme 1). However, due to the lower overall yield of the synthesis (~50%) and the availability of jervine, there is still a strong need of a significantly more efficient method to obtain cyclopamine. Peimisine is a natural product mainly existed into Fritillaria plants which are widely cultivated in China and heavily used in the traditional Chinese medicine [14]. The annual production of Fritillaria bulbs in China is about 5000–6000 t. According to literature, peimisine is the most abundant alkaloid in many Fritillaria bulbs [15], and it can provide sufficient quantity of peimisine if needed.

The structural difference between peimisine and cyclopamine is only at the ring B of the isosteroidal skeleton where peimisine has 6-ketone group while cyclopamine has 5, 6-double bond. Hence, to convert 6-ketone of peimisine into corresponding 5-ene will lead to cyclopamine (Scheme 1). In our study, a conventional conversion involving ketone reduction and elimination of hydroxyl group on ring B didn't give desired result due to the instability of the E/F ring under the reaction conditions [16]. However, a Bamford–Stevens reaction based conversion gave excellent overall yield and very easy work-up process.

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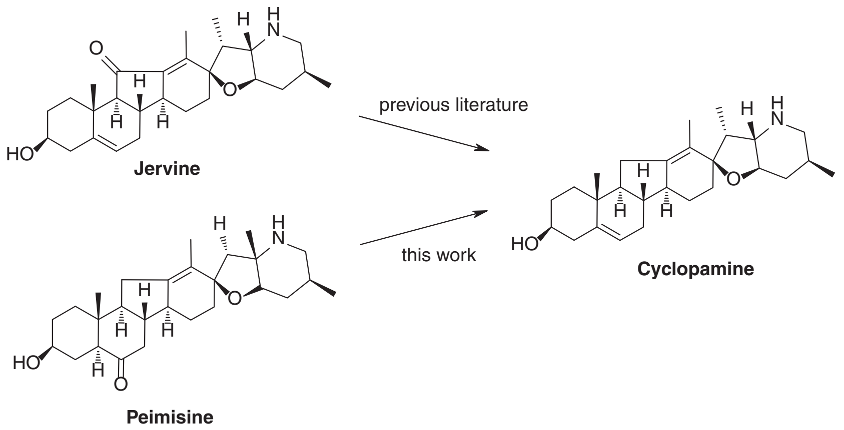

**Scheme 1.** The synthesis of cyclopamine from different approaches.

## 2. Experimental

### 2.1. General

Reagents were purchased from commercial vendors and used as received unless otherwise stated. Toluene (Tol.) was distilled from $CaH_2$. Reactions were monitored by analytical thin-layer chromatography (TLC) with silica gel $GF_{254}$ with a layer thickness of 0.25 mm purchased from Tsingdao Haiyang Co. Ltd. All reactions were stirred with PTFE coated magnetic stir bars and Heidolph® MR3001® stirrers. Removal of solvents was typically accomplished using a rotary evaporator. Mp was checked at Buchi Melting Point B-540. NMR spectra were recorded at 400 MHz with TMS as an internal standard unless otherwise indicated. MS (ESI-MS) was recorded on a Varian 1200L Quadrupole or Agilent 1100 LC/MSD or Waters Acquity SQD MS spectrometer. Chemical shifts are reported in δ, coupling constants in Hz, and IR spectra in $cm^{-1}$.

### 2.2. The synthesis of cyclopamine from peimisine

In a 100-mL round-bottom flask, peimisine (500 mg, 1.17 mmol) and p-tolylsulfonylhydrazine (327 mg, 1.76 mmol) were dissolved in anhydrous methanol (50 mL). p-TSA (402 mg, 2.34 mmol) was added and the mixture was stirred for 4 h at room temperature (about 28 °C). The reaction mixture was concentrated to dryness under reduced pressure to give the crude tosylhydrazone **1** as white solid. This crude intermediate was dissolved in anhydrous toluene (50 mL), and LiH (187 mg, 23.4 mmol) was added. The mixture was refluxed for 5 h under Ar, and filtered after cooled. The filtrate was concentrated to afford the white solid which was recrystallized with acetone and petroleum ether to deliver cyclopamine as white solid (433 mg, 90%). Mp 225–227 °C (lit. [17] 237–238 °C); $^1H$ NMR (400 MHz, $CDCl_3$): $\delta_H$ 0.93 (m, 9H, H-19, 21, 27), 1.63 (s, 3H, H-18), 3.28 (m, 1H, H-23), 3.56 (m, 1H, H-3), 5.36 (brs, 1H, H-6); $^{13}C$ NMR (100 MHz, $CDCl_3$): $\delta_C$ 143.3, 141.8, 126.7, 122.1, 85.8, 75.3, 72.0, 66.3, 54.6, 52.3, 49.5, 42.0, 41.7, 39.7, 39.2, 38.4, 36.8, 32.1, 31.6, 31.4, 31.3, 29.2, 24.7, 19.1, 18.9, 13.3, 11.1; IR (KBr) $\nu_{max}$: 3414, 2928, 1636, 1458, 1122, 1036; (+)-ESIMS m/z, 412.3 $[M+1]^+$.

Intermediate **1** was also obtained as white solid by prep. TLC eluted with ethyl acetate and petroleum ether (1:1, containing 10% triethyl amine). Mp 196–198 °C; $^1H$ NMR (400 MHz, $CDCl_3$): $\delta_H$ 0.34 (s, 3H, H-19), 0.90–0.93 (m, 6H, H-21, 27), 1.54 (s, 3H, H-18), 2.42 (s, 3H, $ArCH_3$), 7.29 (d, 2H, J = 8.0 Hz, ArH), 7.79 (d, 2H, J = 8.0 Hz, ArH); $^{13}C$ NMR (100 MHz,$CD_3$): $\delta_C$ 159.9, 144.2, 141.0, 135.2, 129.7, 128.6, 128.3, 85.2, 71.5, 66.5, 55.0, 54.9, 48.3, 46.4, 44.7, 42.2, 39.8, 39.3, 37.9, 36.9, 31.9, 30.7, 30.6, 30.4, 28.8, 27.2, 25.2, 24.4, 21.8, 19.1, 13.3, 11.6, 10.9; IR (KBr) $\nu_{max}$: 3416, 2928, 2856, 1701, 1637, 1448, 1335, 1165, 1034; (+)-ESIMS m/z 596.4 $[M+1]^+$; HR-ESIMS m/z 596.3512 $[M+1]^+$ (calcd. for $C_{34}H_{50}N_3O_4S_1$ 596.3516).

### 2.3. The synthesis of veraflorizine from peimisine

In a 25-mL round-bottom flask, peimisine (35 mg, 0.08 mmol) and p-tolylsulfonylhydrazine (23 mg, 0.12 mmol) were dissolved into 6 mL of anhydrous methanol. Two drops (about 0.02 mL) of concentrated HCl (37%) were added and the mixture was stirred for 3 h at room temperature (about 28 °C). After removal of solvent under vacuum, the crude tosylhydrazone **2** was obtained as white solid. 6 mL of anhydrous toluene and LiH (13 mg, 1.60 mmol) was added. The mixture was refluxed for 3 h under Ar, and filtered after cooled. The filtrate was concentrated to afford the white solid product. The product was purified over flash chromatography eluted with ethyl acetate and petroleum ether (1:1 containing 10% triethyl amine) to afford veraflorizine as white solid (29 mg, 88%) Mp: 158–160 °C (lit. [18] 175–176 °C); $^1H$ NMR (400 MHz, $CDCl_3$): $\delta_H$ 1.02 (s, 3H, H-21), 1.07 (d, 3H $J = 6.8$ Hz, H-27), 1.56 (s, 3H, H-19), 3.55 (m, 1H, H-3), 5.36 (brs, 1H, H-6); $^{13}C$ NMR (100 MHz, $CDCl_3$): $\delta_H$ 142.1, 122.5, 72.1, 71.2, 70.5, 62.8, 62.0, 54.4, 49.1, 44.8, 42.0, 41.8, 38.9, 38.3, 37.7, 37.1, 31.6, 29.6, 29.4, 27.9, 25.3, 20.9, 20.5, 19.3, 19.2, 17.5; IR (KBr) $\nu_{max}$: 3414, 2934, 2903, 2845, 16,427, 1464, 1377, 1055; (+)-ESIMS m/z 414.3 $[M+1]^+$; HR-ESIMS m/z 414.3359 $[M+1]^+$ (calcd. for $C_{27}H_{44}N_1O_2$ 414.359).

The intermediate tosylhydrazone **2**: White solid, Mp: 200–202 °C; $^1H$ NMR (400 MHz, $CD_3OD$): $\delta_H$ 0.62 (s, 3H, H-19), 1.13 (d, 3H, $J = 7.2$ Hz, H-27), 1.16 (s, 3H, H-21), 3.48 (m, 1H, H-3), 5.36 (brs, 1H, H-6); 7.35 (d, 2H, $J = 8.0$ Hz, ArH), 7.79 (d, 2H, $J = 8.0$ Hz, ArH); $^{13}C$ NMR (100 MHz, acetone-$d_6$): $\delta_C$ 160.8, 143.4, 136.9, 129.3, 128.2, 70.6, 62.4, 61.8, 57.1, 51.6, 49.2, 46.3, 44.0, 40.8, 40.2, 39.5, 37.6, 36.8, 32.3, 31.2, 30.9,