Derivative Synthesis of Wanpeinine A, a Major Steroidal Alkaloid from *Fritillaria shuchengensis*

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Abstract: **Objective** To design and synthesize derivatives of wanpeinine A, the main steroidal alkaloid isolated from the plant *Fritillaria shuchengensis*, and further study on the structure-activity relationship of the steroidal alkaloid. **Methods** Acylation and alkylation were used to synthesize the derivatives and their structures were identified *via* NMR and MS. **Results** The acylation of wanpeinine A (**1**) produced 3β,6α-diacetylwanpeinine A (**2**), 3β,6α-dipropionyl-wanpeinine A (**3**), 3β,6α-dichloracetylwanpeinine A (**4**), 3β,6α-dibenzoylwanpeinine A (**5**)**,** and 3β-methoxy-acylwanpeinine A (**6**). The alkylation of wanpeinine A formed 3β,6α-dimethoxymethylwanpeinine A (**7**). **Conclusion** All compounds are new except for 3β,6α-diacetylwanpeinine A .

Keywords: *Fritillaria shuchengensis*; steroid alkaloid; wanpeinine A

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Introduction

Fritillaria shuchengensis S. C. Chen et S. F. Yin, a well-known medicinal plant that can be found in Shucheng, Anhui Province, China, has been used as an antitussive agent (Zhang, Li, and Chen, 1993). Pharmacological effects of steroid alkaloids in species of *Fritillaria* L. were well documented for their antiasthmatic and antitussive activity, anti-cholinergic activity, and antitumour activity (Yu *et al*, 2000; Zhu and Liao, 2008). Over the years, efforts to study the pharmacological mode of *F. shuchengensis* action were made to isolate and identify its active ingredients. For example, investigations through photochemistry approaches led to the isolation of C-nor-D-homo steroidal alkaloids, which contained the mixture of veraflorizine, ebeinone, verticinone, and isoverticine (Wang and Zhou, 2008). On the other hand, in the course of our investigation on *F. shuchengensis*, we isolated another steroid alkaloid and identified it as wanpeinine A (Li and Wu, 1986). This discovery indicated that wanpeinine A could be a vital component in the plant contributing to its pharmacological effects.

From pharmacological and medicinal chemistry points of view, isolation and structural elucidation of each steroid alkaloid are important to identify the active ingredients of *F. shuchengensis*. Meanwhile, synthetic derivatives of natural steroid alkaloids could help researchers to understand the structural activity relationship (SAR) of each class of steroid alkaloids as well. Success in this field included the structural characterization of wanpeinine A, 5α,14α,22β-cevanine-3β,6α,20β-triol, was the main steroidal alkaloid from *F. shuchengensis* (Li, Jiang and Li, 2006) (Fig. 1). During our investigation, this molecule showed poor liposolubity. Upon a close look, we envisioned the modification of the three hydroxy groups presented within the molecule could potentially lead to the improvement of both physico-chemical property and the DMPK profile of the molecule. In addition, this medicinal chemistry approach could also lay the foundation for future SAR exploration of this class of steroidal alkaloids. To the best of our knowledge, there is no literature precedence in this research field. Herein, we report our research results of synthesis and structural elucidation of wanpeinine A through acylation and alkylation.

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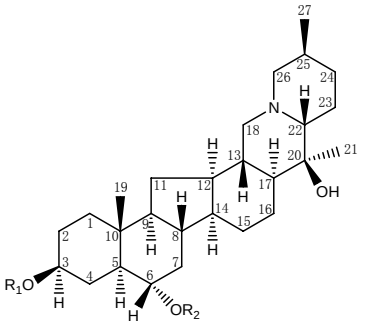

	R_1 (')position)	R_2 (')position)
1	H	H
2	CH_3CO	CH_3CO
3	CH_3CH_2CO	CH_3CH_2CO
4	CH_2ClCO	CH_2ClCO
5	PhCO	PhCO
6	CH_3OCO	H
7	CH_3OCH_2	CH_3OCH_2

Fig. 1 Structures of compounds 1–7

Experiment

General

NMR spectra were recorded on Bruker AC-E 200 and Varian Unity INOVA 400/54 NMR spectrometers with TMS as an internal standard; IR was measured with a ThermoFisher Nicolet 6700 spectrometer; ESI-MS and HR-ESI-MS were carried out on a VG Auto Spec 3000 or Finnegan MAT 90 instrument in positive ion mode; Silica gels were offered by Qingdao Haiyang Chemical Co., Ltd.; All chemical reagents were purchased from Chengdu HengXin Chemical Reagent Co., Ltd.; Acetic anhydride, chloroform, and pyridine were redistilled prior to use. DMF was treated *via* reduced pressure distillation with anhydrous $CaSO_4$.

Sample source

F. shuchengensis was purchased from the Chinese medicinal materials market in the city of Bozhou, Anhui Province, China, July, 2006 and authenticated by LI Ping, a professor in School of Life Science and Engineering, Southwest Jiaotong University, where a voucher specimen was deposited.

Extraction, isolation, and identification

Pulverized dry bulbs of *F. shuchengensis* (10 kg) were extracted with aqueous HCl (0.05 mol/L), and subjected to strongly acidic cationic exchange resins (The resins were washed to neutral with distilled water, dried in the shade, and then alkalified with 10% $NH_3 \cdot H_2O$) using $CHCl_3$ and EtOH as developing elute. As a result, total alkaloids Ⅰ (45.8 g) and Ⅱ (0.35 g) were obtained.

Total alkaloid Ⅰ was chromatographed on a silica gel H column eluting with the petroleum ether/acetone (gradient 50∶1–1∶1) to get four fractions A–D. Fraction C was subjected to silica gel H column chromatography eluting with chloroform/methanol (20∶1) to afford **1** (370 mg), meanwhile **1** (505 mg) was obtained via recrystallization of fraction D.

Wanpeinine A **(1)**: White cluster (methanol), mp 281–283 ℃, ESI-MS *m/z*: 432.5 $[M + 1]^+$, $C_{27}H_{45}NO_3$; $IR\nu_{max}^{KBr}$ (cm^{-1}): 3381, 3294, 1055, 1043 (-OH), 2928, 2905, 2858, 1471, 1446, 1421 (-CH_3, -CH_2-), the IR spectra showed no absorption bands at (2800–2700) cm^{-1} characteristic to *trans*-quinolizidine moiety. So the junction of E and F rings was *cis*, different from most steroid alkaloids in stereo chemical structure. ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 0.74 (3H, s, H-19), 1.09 (3H, s, H-21), 3.39 (1H, m, H-6), 3.41 (1H, m, H-3); ^{13}C-NMR (100 MHz, DMSO-d_6) δ: 37.7 (C-1), 31.1 (C-2), 70.0 (C-3), 32.6 (C-4), 51.9 (C-5), 69.8 (C-6), 40.4 (C-7), 36.2 (C-8), 56.7 (C-9), 34.8 (C-10), 28.7 (C-11), 39.6 (C-12), 38.1 (C-13), 43.1 (C-14), 24.1 (C-15), 19.9 (C-16), 47.0 (C-17), 60.5 (C-18), 12.9 (C-19), 70.4 (C-20), 21.8 (C-21), 68.6 (C-22), 17.8 (C-23), 27.4 (C-24), 25.9 (C-25), 59.4 (C-26), 17.0 (C-27). Compound **1** was identified as wanpeinine A by comparison with the literature reported (Li and Wu, 1986).

Preparation of derivatives

3β,6α-Diacetylwanpeinine A (2) Wanpeinine A (80 mg, 0.184 mmol), pyridine (3 mL), and redistilled acetic anhydride (20 mL, 212 mmol) were added to a 50 mL, round-bottomed flask. The reaction mixture was stirred at room temperature for 48 h. The reaction was quenched with ice. The resulting solution was then adjusted to pH 10 with 10% sodium hydroxide aqueous solution. The reaction mixture was then extracted with chloroform (3 × 10 mL) and dried over Na_2SO_4. The organic solvent was evaporated under vacuum. The crude product was purified by column chromatography (silica gel, chloroform-methanol 120∶1) to give the desired product as a beige solid (21 mg, yield 22%). ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 0.88 (3H, s, H-19), 1.04 (3H, s, H-21), 1.08 (3H, d, J = 7.6 Hz, H-27), 2.01 (6H, s, H-2′ and H-2″), 4.66 (2H, m, H-3 and H-6); ^{13}C-NMR (50 MHz, $CDCl_3$) δ: 37.2 (C-1), 29.2 (C-2), 73.2 (C-3), 29.6 (C-4), 48.9 (C-5), 73.0 (C-6), 40.9 (C-7), 36.5 (C-8), 56.4 (C-9), 35.3 (C-10), 28.3 (C-11), 40.9 (C-12), 38.8 (C-13), 43.3 (C-14), 24.6 (C-15), 20.5 (C-16), 48.7 (C-17), 61.7 (C-18), 12.8 (C-19), 70.5 (C-20), 20.5 (C-21), 70.9 (C-22), 18.9 (C-23), 27.5 (C-24), 26.8 (C-25), 62.1 (C-26), 17.3 (C-27), 170.7 (C-1′), 21.3 (C-2′), 170.5 (C-1″), 21.2 (C-2″). The ^{1}H-NMR data of

compound **2** coincided with the data in literature (Li and Wu, 1986).

3β,6α-Dipropionylwanpeinine A (3) Wanpeinine A (43.1 mg, 0.100 mmol) in 2 mL of chloroform was added in a 10 mL test tube, followed by the addition of propionyl chloride (0.026 mL, 0.300 mmol) dropwise. After the addition, the reaction mixture was stirred at room temperature for another 8 h. After the reaction was quenched by ice, the mixture was extracted with chloroform (3 × 8 mL). The combined organic phase was washed with the saturated solution of sodium bicarbonate (3 × 8 mL) and water (3 × 10 mL), dried over Na_2SO_4 and concentrated under vacuum. The residue was purified by centrifugal chromatography (chloroform-methanol 150∶1) to give the desired product as a yellow solid (15 mg, yield 28%). HR-ESI-MS *m/z*: 544.3992 $[M+H]^+$, calcd for $C_{33}H_{54}NO_5$ $[M+H]^+$, 544.4002. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 0.89 (3H, s, H-19), 1.02 (3H, s, H-21), 1.08 (3H, d, J = 7.8 Hz, H-27); ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 37.3 (C-1), 29.3 (C-2), 73.0 (C-3), 29.4 (C-4), 48.9 (C-5), 72.8 (C-6), 37.3 (C-7), 36.6 (C-8), 56.5 (C-9), 35.4 (C-10), 28.4 (C-11), 39.0 (C-12), 39.1 (C-13), 43.4 (C-14), 24.7 (C-15), 20.6 (C-16), 48.8 (C-17), 61.7 (C-18), 12.7 (C-19), 70.9 (C-20), 20.3 (C-21), 70.3 (C-22), 19.0 (C-23), 26.9 (C-24), 27.5 (C-25), 62.4 (C-26), 17.2 (C-27), 174.1 (C-1′), 27.9 (C-2′), 9.2 (C-3′), 173.9 (C-1″), 27.8 (C-2″), 9.1 (C-3″).

3β,6α-Dichloracetylwanpeinine A (4) Wanpeinine A (43.1 mg, 0.100 mmol) and DMF (2 mL) were added in a 10 mL test tube equipped with magnetic stir bar in an ice bath, followed by the addition of sodium hydride (72 mg, 0.300 mmol). The reaction mixture was stirred at 0 ℃ for 4 h. Chloroacetyl chloride (0.023 mL, 0.300 mmol) in 2 mL of DMF was added dropwise to the solution and stirred 0 ℃ for another 6 h. TLC was used to monitor the reaction progress. At the end, the reaction was quenched by ice and the reaction mixture was extracted with chloroform (3 × 8 mL). Then the organic layers were combined, washed with the saturated solution of sodium bicarbonate (3 × 8 mL) and water (3 × 10 mL), dried over Na_2SO_4, and evaporated in vacuum. The residue was purified by column chromatography on silica gel (1 cm × 15 cm, chloroform-methanol 100∶1) to give the compound **6**, as a yellow solid (15 mg, yield 26%). HR-ESI-MS *m/z*: 584.2903 $[M+H]^+$, calcd for $C_{31}H_{48}NO_5Cl_2$ $[M+H]^+$, 584.2910. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 0.91 (3H, s, H-19), 1.03 (3H, s, H-21), 1.08 (3H, d, J = 7.8 Hz, H-27), 4.03 (2H, s, H-2″), 4.04 (2H, s, H-2′); ^{13}C-NMR (50 MHz, $CDCl_3$) δ: 37.1 (C-1), 31.7 (C-2), 75.4 (C-3), 32.7 (C-4), 48.8 (C-5), 75.2 (C-6), 40.9 (C-7), 36.3 (C-8), 56.3 (C-9), 35.4 (C-10), 28.1 (C-11), 39.1 (C-12), 38.6 (C-13), 43.5 (C-14), 24.6 (C-15), 20.6 (C-16), 48.8 (C-17), 61.7 (C-18), 12.9 (C-19), 70.9 (C-20), 22.6 (C-21), 70.3 (C-22), 19.0 (C-23), 27.6 (C-24), 26.6 (C-25), 62.2 (C-26), 17.2 (C-27), 188.9 (C-1′), 41.1 (C-2′), 186.7 (C-1″), 41.0 (C-2″).

3β,6α-Dibenzoylwanpeinine A (5) This compound was prepared under the same condition as compound **4** except that chloroacetyl chloride was replaced by benzoyl chloride (0.051 mL, 0.300 mmol) and the final product was purified on silica gel column (1 cm × 15 cm, chloroform-methanol 160∶1) to give the desired product as a yellow solid (11 mg, yield 17%). HR-ESI-MS *m/z*: 640.3997 $[M+H]^+$, calcd for $C_{41}H_{54}NO_5$ $[M+H]^+$, 640.4002. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 0.88 (3H, s, H-19), 1.11 (3H, s, H-21), 7.38–7.49 (4H, m, H-4′, H-6′, H-4″, and H-6″), 7.45–7.54 (2H, m, H-5′, and H-5″), 7.98–8.05 (4H, m, H-3′, H-7′, H-3″, and H-7″); ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 37.1 (C-1), 31.9 (C-2), 74.0 (C-3), 32.7 (C-4), 49.3 (C-5), 73.8 (C-6), 40.9 (C-7), 36.6 (C-8), 56.4 (C-9), 35.6 (C-10), 28.6 (C-11), 38.7 (C-12), 37.3 (C-13), 43.4 (C-14), 24.5 (C-15), 19.7 (C-16), 48.6 (C-17), 61.6 (C-18), 12.8 (C-19), 71.0 (C-20), 22.7 (C-21), 71.0 (C-22), 18.8 (C-23), 27.4 (C-24), 27.0 (C-25), 56.6 (C-26), 17.4 (C-27), 166.2 (C-1′), 130.6 (C-2′), 129.5 (C-3′ and C-7′), 128.3 (C-4′ and C-6′), 132.8 (C-5′), 166.0 (C-1″), 130.4 (C-2″), 129.5 (C-3″ and C-7″), 128.2 (C-4″ and C-6″), 132.7 (C-5″).

3β-Methoxyacylwanpeinine A (6) This compound was synthesized under the same condition as compound **4** except that chloroacetyl chloride was replaced by methyl chloroformate (0.023 mL, 0.300 mmol) and the final product was purified by silica gel column chromatography (1 cm × 15 cm, chloroform-methanol 100∶1) to give the desired product as a beige solid (12 mg, yield 25%). HR-ESI-MS *m/z*: 490.3541 $[M+H]^+$, calcd for $C_{29}H_{48}NO_5$ $[M+H]^+$, 490.3532. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 0.83 (3H, s, H-19), 1.03 (3H, s, H-21), 3.51 (3H, s, H-2′); ^{13}C-NMR (100 MHz, $CDCl_3$)

δ: 37.4 (C-1), 31.9 (C-2), 77.2 (C-3), 32.3 (C-4), 52.0 (C-5), 70.2 (C-6), 40.8 (C-7), 37.3 (C-8), 54.5 (C-9), 33.7 (C-10), 28.0 (C-11), 40.3 (C-12), 38.6 (C-13), 43.4 (C-14), 24.4 (C-15), 19.7 (C-16), 48.5 (C-17), 61.9 (C-18), 12.8 (C-19), 71.1 (C-20), 22.7 (C-21), 70.2 (C-22), 17.6 (C-23), 27.1 (C-24), 26.7 (C-25), 56.7 (C-26), 17.1 (C-27), 155.3 (C-1′), 56.7 (C-2′).

3β,6α-Dimethoxymethylwanpeinine A (7) This compound was synthesized under the same condition as compound **4** except that chloroacetyl chloride was replaced by chloromethyl methyl ether (0.023 mL, 0.300 mmol) and the final product was purified by silica gel column chromatography (1 cm × 15 cm, chloroform-methanol 120∶1) to give the desired product as a yellow solid (11 mg, yield 22%). HR-ESI-MS *m*/*z*: 520.4001 $[M + H]^+$, calcd for $C_{31}H_{54}NO_5$ $[M + H]^+$, 520.4001. ^{1}H-NMR (400 MHz, $CDCl_3$) *δ*: 0.74 (3H, s, H-19), 1.02 (3H, s, H-21), 1.08 (3H, d, *J* = 8.0 Hz, H-27), 3.36 (3H, s, H-2″), 3.37 (3H, s, H-2′); ^{13}C-NMR (50 MHz, $CDCl_3$) *δ*: 37.3 (C-1), 29.4 (C-2), 71.0 (C-3), 39.7 (C-4), 50.5 (C-5), 70.4 (C-6), 41.1 (C-7), 35.3 (C-8), 56.7 (C-9), 31.9 (C-10), 28.1 (C-11), 39.7 (C-12), 39.1 (C-13), 43.5 (C-14), 24.8 (C-15), 19.0 (C-16), 48.8 (C-17), 61.8 (C-18), 12.8 (C-19), 68.3 (C-20), 22.6 (C-21), 68.1 (C-22), 17.3 (C-23), 27.7 (C-24), 26.7 (C-25), 61.8 (C-26), 14.1 (C-27), 95.5 (C-1′), 55.5 (C-2′), 94.5 (C-1″), 55.2 (C-2″).

Further research of pharmacological activity of the derivatives is under going.

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