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Synthesis, isolation, stereostructure and cytotoxicity of paclitaxel analogs from cephalomannine

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ABSTRACT

Four paclitaxel derivatives were afforded by preparative HPLC separation of two pairs of diastereoisomers, which were obtained by catalytic hydrogenation and epoxidation of the C-13 side-chain double bond of cephalomannine, a naturally occurring paclitaxel analog. The four paclitaxel derivatives were analyzed using NMR, CD spectroscopy, and side-chain hydrolysis in order to measure their optical rotations and GC characteristics. In this way, the stereoconfigurations of the products were determined. Evaluation of the compounds' activity indicated that they had differing cytotoxic activities: compound **5** had superior activity in BCG-823 tumor cells compared to paclitaxel, while compound **7** had superior activity in HCT-8 and A549 tumor cells compared to paclitaxel. These results indicate that the stereoconfiguration of the paclitaxel *N*-acyl side chain has a significant impact on its activity.

1. Introduction

Paclitaxel, or Taxol® (**1**, Fig. 1), is a structurally peculiar diterpenoid compound that was isolated from the bark of the North American *Taxus brevifolia* by Wani et al. in 1971 [1]. Because of its significant anticancer activity, unique mechanism of action, complex molecular structure, and multiple chiral centers, research on its chemical synthesis, structural modification, and structure–activity relationship has long been a focus in the field of natural product chemistry [2]. At present, paclitaxel and its semisynthetic derivative, docetaxel (**2**, Fig. 1), are mainly used as clinical treatments for breast, ovarian, and non-small-cell lung cancer [3].

As can be seen from its name, cephalomannine (**3**, Fig. 1) was initially considered to have been isolated from *Cephalotaxus mannii*, but it was later discovered that there was an error in the plant classification and that cephalomannine was actually from *Taxus wallichiana*, a species of yew [4]. In later phytochemical studies, cephalomannine was found in several other yew species [5–7]. The structure of cephalomannine is very similar to that of paclitaxel, differing only in its C-13 side chain: paclitaxel has an *N*-benzoyl group in its C-13 side chain, while cephalomannine has an *N*-tigloyl group. Because of these structural similarities, the separation of cephalomannine from paclitaxel is very difficult, and several methods for effectively separating the two compounds have been developed [8–10]. Since the amount of cephalomannine might even be higher than that of paclitaxel in certain *Taxus* plants, it can also be a useful semi-synthetic raw material that can be converted into paclitaxel or docetaxel [11,12]. Furthermore, there have been reports of interesting structural modifications and biotransformations of cephalomannine [13–15]. Therefore, structural modification of readily available cephalomannine can contribute to a better understanding of the structure–activity relationship in the anticancer effects of taxanes.

The epoxidation of cephalomannine has been reported in a patent on the preparation of cephalomannine analogs, but the diastereomers were not separated in this report [16]. There is no stereoselectivity of hydrogenation and epoxidation reactions on the double bond of cephalomannine's side-chain and the afforded compounds in the patent were a pair of diastereoisomers. Therefore, in the current study, catalytic hydrogenation and epoxidation of cephalomannine were carried out separately on the side-chain *N*-tigloyl double bond to obtain two different diastereoisomers, which were then

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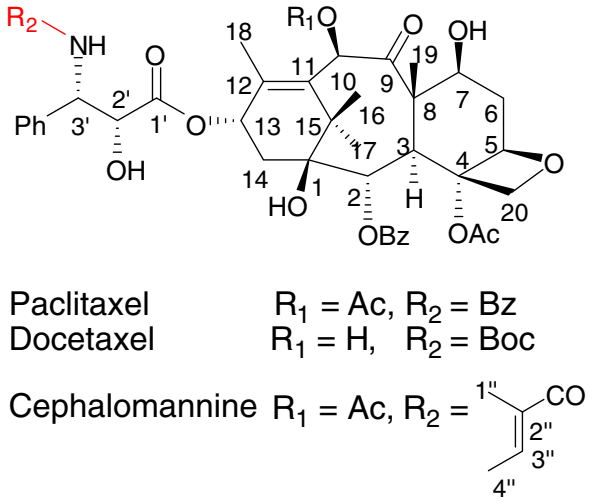

Fig. 1. Structures of paclitaxel (**1**), docetaxel (**2**) and cephalomannine (**3**).

separated using preparative high-performance liquid chromatography (HPLC). In addition, the three-dimensional structures as well as anticancer activities of the obtained diastereoisomers were studied.

2. Experimental

2.1. General

^{1}H and ^{13}C NMR spectra were obtained on a Varian Unity INOVA 400/54 and Bruker ARX-500 spectrometer in $CDCl_3$ with TMS as the internal standard. Mass spectra were obtained on a VG Auto spec 3000 or on a Finnigan MAT90 instrument. Optical rotations were measured on a Perkin-Elmer 341, and CD spectra were measured on a JASCO J-810 spectropolarimeter. Preparative HPLC was performed on a Shimadzu LC-8A liquid chromatograph equipped with a Zorbax PrepHT GF (21.2 mm × 25 cm, 7 μm) or Venusil MP C18 (20 mm × 25 cm, 5 μm) column. GC–MS analysis was performed on a GC (Agilent, 6890N) interfaced with a mass selective detector (Agilent, 5973B). Silica gel H (Qingdao Sea Chemical Factory, Qingdao, People's Republic of China) was used for column chromatography.

Spots on TLC (silica gel G) were detected by spraying with H_2SO_4–EtOH. Commercially available reagents and solvents were directly used without further purification.

2.2. Substrates

Cephalomannine (**3**) was purchased from Kunming Wuyi Biotechnology Co., Ltd., China. The structures were characterized on the basis of ^{1}H NMR and ^{13}C NMR spectra, and the purities were determined to be >95% by HPLC analyses.

2.3. Preparation of compounds **4** and **5**

A solution of cephalomannine (200 mg, 0.24 mmol) and Pd–C (10 mg, 5%) under H_2 atmosphere was strongly stirred for 24 h. Then the reaction solution was filtered and evaporated to give a mixture of compounds **4** and **5**. The mixture was further subjected to preparative reversed-phase HPLC eluting with CH_3CN–H_2O (60/40, v/v) to give compounds **4** (85 mg, t_R = 16.7 min) and **5** (82 mg, t_R = 18.6 min).

2.3.1. N-debenzoyl-N-(S-2-methylbutanoyl)paclitaxel (**4**)

White powder, $[a]_D^{25}$ − 49 ° (c 0.10, MeOH); IR ν_{max} 3438, 2961, 1730, 1714, 1638 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$): see Table 1. MS (ESI, MeOH) *m/z* 834 $[M + H]^+$.

2.3.2. N-debenzoyl-N-(R-2-methylbutanoyl)paclitaxel (**5**)

White powder, $[a]_D^{25}$ − 25 ° (c 0.10, MeOH); IR ν_{max} 3438, 2962, 1730, 1716, 1638 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$): see Table 1; MS (ESI, MeOH) *m/z* 834 $[M + H]^+$. HR-ESI-MS: 834.3632 $[M + H]^+$ (calcd. for $C_{45}H_{56}NO_{14}$ 834.3622).

2.4. Preparation of compounds **6** and **7**

The solution of cephalomannine (250 mg, 0.30 mmol) in CH_3Cl_3 (50 mL) was added in *m*CPBA (3-chloroperbenzoic acid, 62 mg, 0.36 mol, 1.2 equiv.). The reaction mixture was stirred for 8 h under argon prior to being quenched with aqueous solution of $Na_2S_2O_3$. The organic phase was separated, washed with brine and dried over anhydrous Na_2SO_4. Then the organic solvent was removed under reduced pressure to give the mixture of compounds **6** and **7**. The mixture was further subjected to preparative reversed-phase HPLC eluting with CH_3CN–H_2O (60/40, v/v) to give compounds **6** (76 mg, t_R = 12.7 min) and **7** (72 mg, t_R = 15.2 min).

2.4.1. N-debenzoyl-N-[(2R,3S)-2,3-epoxy-2-methylbutanoyl] paclitaxel (**6**)

White powder, $[a]_D^{25}$ − 38 ° (c 0.10, MeOH); IR ν_{max} 3422, 2988, 1710, 1632 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$): see Table 1; MS (ESI, MeOH) *m/z* 848 $[M + H]^+$. HR-ESI-MS: 848.3466 $[M + H]^+$ (calcd. for $C_{45}H_{54}NO_{15}$ 848.3415).

2.4.2. N-debenzoyl-N-[(2S,3R)-2,3-epoxy-2-methylbutanoyl] paclitaxel (**7**)

White powder, $[a]_D^{25}$ − 66 ° (c 0.10, MeOH); IR ν_{max} 3422, 2988, 1712, 1632 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$): see Table 1; MS (ESI, MeOH) *m/z* 848 $[M + H]^+$. HR-ESI-MS: 848.3446 $[M + H]^+$ (calcd. for $C_{45}H_{54}NO_{15}$ 848.3415).

2.5. Complete alkaline hydrolysis of compounds **4**–**7**

A solution of **4** (or **5**, **6**, **7**) (50 mg) and KOH (100 mg) in MeOH (15 mL) was stirred overnight at room temperature. The reaction mixture was neutralized with 1 N HCl and extracted with EtOAc (10 mL × 3). Then the combined organic phase was dried over anhydrous Na_2SO_4 and evaporated to give the residue. The two residues, from compounds **4** and **5**, were dissolved in MeOH (5 mL) and treated with excess CH_2N_2. After evaporation, the residues were dissolved in $CHCl_3$ (5 mL) and filtered. An aliquot of the two filtrates was analyzed by GC using a chiral column (Astec Chiraldex G-TA G0012-08, 30 m, 50 °C), to give peaks at t_R 12.5 or 11.4 min, respectively. The standard methyl ester of (*R*)-or (*S*)-2-methylbutyric