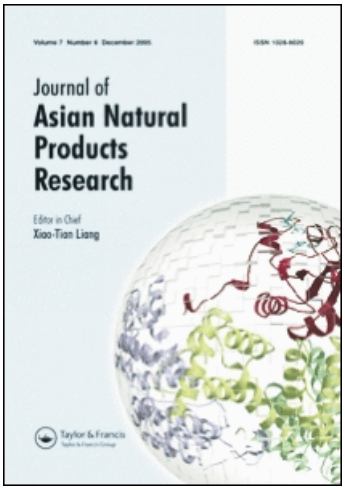

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Preparation and evaluation of new brominated paclitaxel analogues

Yi Jiang[a]; Hai-Xia Lin[a]; Ming Li[a]; Bei-Li Wu[a]; Jian-Min Chen[b]
[a] †Department of Environmental Science and Engineering, Fudan University, Shanghai, China [b] ‡Ministry of Education Key Laboratory for Biodiversity Science and Ecological Engineering, Institute of Biodiversity Science, school of Life Science, Fudan University, Shanghai, China

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Preparation and evaluation of new brominated paclitaxel analogues

YI JIANG†, HAI-XIA LIN†, MING LI†, BEI-LI WU† and JIAN-MIN CHEN†‡*

†Department of Environmental Science and Engineering, Fudan University, Shanghai 200433, China
‡Ministry of Education Key Laboratory for Biodiversity Science and Ecological Engineering, Institute of Biodiversity Science, School of Life Science, Fudan University, Shanghai 200433, China

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Two diastereomers of 2″,3″-dibromo-7-epi-10-deacetylcephalomannine, **4** and **5**, have been synthesized, purified and identified for evaluation as antitumour drugs. The cytotoxicity of the two diastereomers, assessed in cell culture against MCF-7 breast cancer, A549 lung cancer and A2780 ovarian cancer, was slightly stronger than that of paclitaxel. The cytotoxicity of **5** outweighs that of **4**. In the light of the difference in cytotoxicity between the two diastereomers, we can assume that the differing configurations of C-2″ and C-3″ of the two diastereomers may result in different bioactive conformations in solution and, consequently, different biologically relevant conformations for binding to tubulin/microtubules — a matter we are studying further.

Keywords: 2″,3″-Dibromo-7-epi-10-deacetylcephalomannine; Bioactive conformation; Brominated paclitaxel analogues; *In vitro* cytotoxicity

1. Introduction

Despite paclitaxel's (**1**) (figure 1) prospects as an anticancer agent and the global excitement surrounding its potential therapeutic value [1–3], several problems associated with the drug hamper its widespread usage. Central among them is the issue of frequent con-occurrence of other taxanes and subsequent resource deficiency. Of all these congeners, cephalomannine (**2**) [4–6] has been taken as the most stubborn during the chromatographic purification process because of the nuance in the end of C-13 side-chain between cephalomannine and paclitaxel, which is an unsaturated double bond in cephalomannine while a phenyl group in the latter. Bromination of cephalomannine was first introduced by Kingston [7] in 1996 to eliminate cephalomannine and harvest pure taxol. Pandey [8,9] thoroughly investigated the products of bromination of cephalomannine, two diastereomers of 2″,3″-dibromocephalomannine, and revealed them as paclitaxel-like antitumor agents both *in vitro* and *in vivo*. Such an exciting result established another structure–activity relationship (SAR), i.e. that bromination of the unsaturated double bond of the C-13 side-chain of cephalomannine and other cephalomannine analogues greatly enhances antitumor activities.

Despite this SAR, however, there are no studies on how the discrepancy between configurations of both C-2″ and C-3″ of the two brominated diastereomers influences their

*Corresponding author. Tel.: +86-21-6564 2521. Fax:+86-21-6564 2080. E-mail: jmchen@fudan.ac.cn

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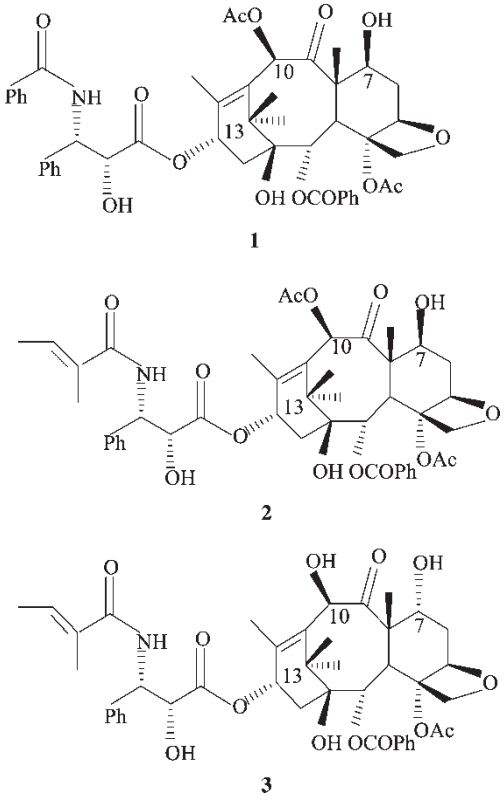


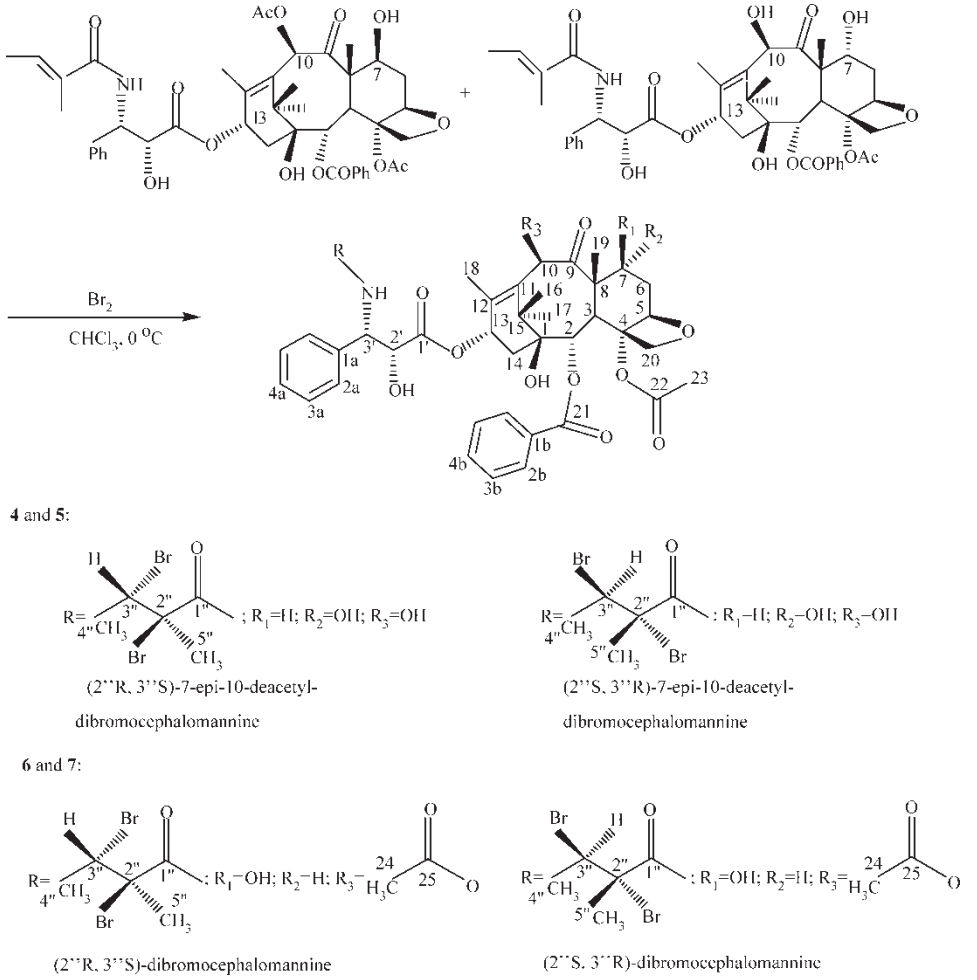
Figure 1. Structures of compounds **1**–**3**.

respective bioactive conformations in solutions, by which we may predict potential models for the biologically relevant conformations for binding to tubulin/microtubules [10]. It is also unknown whether, when the two diastereomers are used together, there is mutual promotion or counteraction effect between the two brominated diastereomers when binding to tubulin/microtubules. Accordingly, we decided to perform NMR studies on the solution structures of the two brominated diastereomers of another cephalomannine analogue, 7-epi-10-deacetylcephalomannine (**3**) [11,12], which only differs from cephalomannine in the group at C-10 and the configuration of the OH group at C-7, and is another closely eluting analogue to paclitaxel in the bark extract of *Taxus yunnanesis*. We report here the first part of our studies, namely the synthesis, purification and evaluation of two diastereomers of 2″,3″-dibromo-7-epi-10-deacetylcephalommannine (scheme 1). Antitumor tests of the two diastereomers of 2″,3″-dibromo-7-epi-10-deacetylcephalomannine against ovarian and breast cancer also showed paclitaxel-like activity, thereby providing another possible alternative to paclitaxel. Also, the conformational explanation of the difference in cytotoxicity between the two diastereomers in the next step will shed more light on the SAR.

2. Results and Discussion

2.1 Stereochemistry of 4 and 5

An usual stereoselective anti-addition mechanism has been applied to explain the bromination of cephalomannine and 7-epi-cephalomannine, respectively, by Kingston [7]



Scheme 1. Bromination of cephalomannine and 7-epi-10-deacetylcephalomannine.

and Pandey [8,9]. Considering that **3** has the same N-tigloyl moiety in the C-13 side-chain as that of **2**, we can conclude that bromination of **3** will also yield two diastereomers of **4** and **5** based on the stereoselective anti-addition mechanism, and that **4** and **5** must differ in their absolute configuration at C-2″ and C-3″. Figure 2 shows that two main compounds were eluted in turn during the semi-preparative separation of reaction products of **3**, which validates the above conclusion.

Despite this, to date, no unambiguous stereochemical assignments have been made for the bromination products of cephalomannine and its analogues such as **4** and **5**. Pandey [8] indicated that the stereochemistry at C-2″ and C-3″ of bromination products of cephalomannine, **6** and **7**, could be interchanged. Indeed, the single bonds connecting C-2″ and C-3″ in the two diastereomers are prone to rotate under specific conditions, which may cause an interchange of the absolute configuration of C-2″ and C-3″. However, various significant discrepancies between **4** and **5**, such as the colour in solid state and crystallization properties, lead us to hesitate in accepting Pandey's conclusion here. Many means, such as X-ray single crystal diffraction and conformation studies in solution using NMR, in conjunction with molecular modelling, are currently being employed in our laboratory to

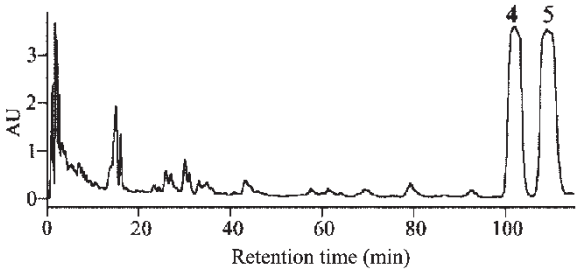


Figure 2. Chromatogram of semi-preparative separation of the two diastereomers of 2″,3″-dibromo-7-epi-10-deacetylcephalomannine.

assign the stereochemistry of **4** and **5** unambiguously, and encouraging progress has been made. The final results will be reported elsewhere in the near future.

2.2 In vitro *cytotoxicity of 4 and 5*

As shown in table 1, the IC%s of both **4** and **5** are higher than that of **1** against all three cell lines used in this study. Compared with MCF-7 and A549, A2780 was the most sensitive to **1**, **4**, and **5** by IC_{50}. By comparing the IC_{50}s of **1**, **4**, and **5** against A2780, we can conclude that, for A2780, **4** has antitumor activity that is at least equal to that of **1** while the antitumor cytotoxicity of **5** is approximately 4 times that of **1**; therefore, the different configurations of C-2″ and C-3″ of both **4** and **5** must be responsible for this discrepancy in *in vitro* cytotoxicity.

Another significant result (table 1) is that although the cytotoxicities of **4** and **5** against A2780 are greater than that of **1**, the 1:1 mixture of **4** and **5** shows lower antitumor activity against A2780 than either **4** or **5**, which supports the assumption that there may exist some counteraction or competitive effect between the two diastereomers when binding to the tubulin/microtubule. Such a result renders our laborious separation work worthwhile and inspires interest in undertaking the conformational analysis of the two drugs in solution in order to elucidate the SAR more clearly.

3. Experimental

3.1 General Experimental Procedures

Semi-purified bark extract containing 14.8% paclitaxel, 16.1% cephalomannine, 24.7% 7-epi-10-deacetyl-cephalomannine and 38.5% 7-epi-10-deacetyl-paclitaxel was purchased from

Table 1. Cytotoxicities of paclitaxel and new brominated analogues in three tumor cell lines (IC_{50}*, $\mu g\,ml^{-1}$ and IC%†).

	MCF-7		*A549*		*A2780*	
Compd	*IC_{50}*	*IC%*	*IC_{50}*	*IC%*	*IC_{50}*	*IC%*
1	>10	30.53	>10	27.37	0.22	60.89
4	>10	44.23	>10	32.56	0.16	64.19
5	>10	45.27	>10	36.18	0.0599	72.94
4 + 5	>10	44.99	>10	36.61	0.445	61.55

* IC_{50} ($\mu g\,ml^{-1}$), concentration required to inhibit cell growth by 50% upon continuous exposure for 72 h.
† IC% = (number of cells whose growth has been inhibited by 10 $\mu g\,ml^{-1}$ of the drug per total number of cells) × 100.

Yunnan Province, China. Acetonitrile and methanol (HPLC grade) and DMSO were purchased from Fisher Scientific Worldwide (Shanghai Representative Office). Ethanol, chloroform, ethyl acetate and n-hexane were analytical grade. Water was purified with a Millipore Milli-Q system. Bromine was obtained from the Shanghai Reagent Company, China.

HPLC analysis was performed on an Agilent 1100 series LC system. LC/MS/MS analysis was performed on a Finnigan LCQ Advantage system with an electrospray interface (ESI). ^{1}H NMR and ^{13}C spectra were recorded with a Bruker DM-500 MHz spectrometer using $CDCl_3$ as internal standard.

3.2 Preparation and purification of 2″,3″-dibromo-7-epi-10-deacetylcephalomannine

Crude plant extract (3.0 g) was dissolved in chloroform so that a total of 250 ml of solution was obtained. To this solution, cooled in an ice bath and continually stirred with a magnetic stirrer, was added chloroform (30 ml). To the cooled solution (0°C), bromine (100 μl) was added dropwise. After 30 min of reaction in the dark, the reaction mixture was then washed with 30 ml 5% aqueous sodium sulfite solution, followed by two washes with distilled water (20 ml each time). The combined aqueous layer was then re-extracted with chloroform (30 ml). The organic layers were combined, dried with anhydrous sodium sulfate (10 g), and evaporated to dryness using a rotary vacuum evaporator at 30°C. The brominated material thus obtained was purified using a normal phase preparative HPLC system with a mobile phase of ethyl acetate-n-hexane (1:1). A total of 30 fractions were collected, and fractions of common ingredients were combined and dried according to the results of reversed-phase analytical HPLC. The dried mixture of **4** and **5** was then loaded onto a reversed-phase semi-preparative HPLC system to obtain a high purity of **4** and **5** individually; **6** and **7** were also purified.

Compound **4**. ^{1}H NMR ($CDCl_3$) (J in Hz) δ (ppm): 1.09 (s, 3H), 1.24 (s, 3H), 1.72 (s, 3H), 1.74 (m, 3H), 1.75 (s, 3H), 1.99 (s, 3H), 2.29 (m, 2H), 2.32 (m, 2H), 2.44 (s, 3H), 3.68 (dd, $J = \mathbf{2.27}$, 2.35, 1H), 3.91 (d, $J = \mathbf{7.37}$, 1H), 4.39 (t, $J = \mathbf{9.39}$, 2H), 4.60 (q, 1H), 4.71 (d, 1H), 4.90 (dd, $J = \mathbf{3.92}$, 3.76, 1H), 5.42 (s, 1H), 5.56 (dd, $J = \mathbf{1.76}$, 1.95, 1H), 5.73 (d, $J = \mathbf{7.37}$, 1H), 6.22 (t, $J = \mathbf{8.45}$, 1H), 7.36–8.12 (m, 11H); ^{13}C NMR ($CDCl_3$) δ (ppm): 14.3, 16.7, 20.5, 22.5, 22.6, 26.0, 26.7, 35.3, 36.3, 40.2, 42.5, 54.3, 55.2, 57.3, 72.7, 72.8, 75.5, 75.8, 76.7, 77.0, 77.2, 79.2, 82.1, 82.6, 126.5, 128.3, 128.7, 129.0, 129.3, 130.1, 133.7, 135.8, 137.3, 137.7, 167.0, 168.7, 172.2, 172.3, 214.9; ESI full MS m/z 972.1 [M + Na].

Compound **5**. ^{1}H NMR ($CDCl_3$) (J in Hz) δ (ppm): 1.09 (s, 3H), 1.23 (s, 3H), 1.72 (s, 3H), 1.73 (m, 3H), 1.74 (s, 3H), 1.94 (s, 3H), 2.29 (m, 2H), 2.31 (m, 2H), 2.46 (s, 3H), 3.68 (dd, $J = \mathbf{2.18}$, 2.60, 1H), 3.91 (d, $J = \mathbf{7.30}$, 1H), 4.39 (t, $J = \mathbf{8.93}$, 2H), 4.60 (q, 1H), 4.72 (d, 1H), 4.89 (dd, $J = \mathbf{3.81}$, 3.76, 1H) 5.43 (s, 1H), 5.58 (dd, $J = \mathbf{1.79}$, 1.96, 1H), 5.72 (d, $J = \mathbf{7.30}$, 1H), 6.24 (t, $J = \mathbf{8.95}$, 1H), 7.36–8.11 (m, 11H); ^{13}C NMR ($CDCl_3$) δ 14.4, 16.7, 20.6, 22.5, 22.7, 26.0, 27.1, 35.3, 36.3, 40.2, 42.5, 54.1, 54.9, 57.2, 72.6, 72.7, 75.5, 75.9, 76.8, 77.0, 77.3, 79.3, 82.1, 82.6, 126.7, 128.4, 128.7, 129.0, 129.3, 130.2, 133.7, 135.7, 137.2, 137.7, 167.1, 168.8, 172.2, 172.3, 214.9; ESI full MS m/z 972.1 [M + Na].

3.3 **In vitro** *cytotoxicity assay*

The activity study was performed by the Shanghai Institute of Medical Industry. The three cell lines used in the evaluation were MCF-7 (breast carcinoma), A2780 (ovarian carcinoma) and P388 (mice leukaemia). They were propagated under sterile conditions in RPMI 1640 with 15% NBS and incubated at 37°C. Four samples of **1**, **4**, **5**, **4** + **5** (1:1) were dissolved in

DMSO and then adjusted to solutions of 100, 10, 1, 0.1, 0.01, and 0.001 $\mu g\,ml^{-1}$ with PBS(−) respectively. The cell lines were treated with the four drugs of different concentrations for 72 h and then processed to analyze for antitumor activity using the microculture tetrozolium (MTT) procedure.

4. Conclusions

We have successfully synthesized and purified two brominated paclitaxel analogues, **4** and **5**, and their structures have been assigned unambiguously based on the 1D and 2D NMR data. Encouraging results have been achieved from the cytotoxicity assay of the two diastereomers against MCF-7 (breast carcinoma), A2780 (ovarian carcinoma) and P388 (mice leukaemia).

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