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Crystallographic determination of stereochemistry of biologically active 2″,3″-dibromo-7-*epi*-10-deacetylcephalomannine

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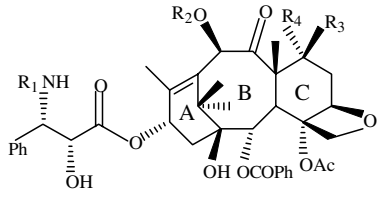
Abstract—The stereochemistry at C2″ and C3″ of two diastereomers of 2″,3″-dibromo-7-*epi*-10-deacetylcephalomannine (**6** and **7**), which were synthesized by reacting 7-*epi*-10-deacetylcephalomannine (**5**) with bromine, were assigned unambiguously based on crystallographic studies of **6**. The X-ray crystallographic analysis shows that **6** adopts an absolute configuration of (2″*S*,3″*R*), and **7** can be assigned as (2″*R*,3″*S*) configuration. The side-chain conformation of **6** was revealed to be different with the known hydrophobic collapse and the apolar conformations, as found in solid state and in solution. However, most side-chain torsion angles of **6** were found to be very similar to those of tubulin-bound T-shaped conformation (T-Taxol). Both **6** and **7** showed strong in vitro paclitaxel-like activity.

Despite paclitaxel's (**1**) prospects as an anticancer agent and the global excitement surrounding its potential therapeutic value,[1,2] several problems associated with the drug hamper its widespread usage. In an ingenious approach to developing a simple and economical method for the separation of paclitaxel and one of its most stubborn congeners, cephalomannine[3] (**2**), Kingston and co-workers[4] converted cephalomannine into two diastereomeric 2″,3″-dibromocephalomannine, which could readily be separated from paclitaxel by chromatography on a Si gel column to yield pure paclitaxel and a mixture of dibrominated compounds. In a complementary study, Pandey et al.[5,6] reported the chromatographic separation of individual diastereomeric 2″,3″-dibromocephalomannine and 2″,3″-dibromo-7-*epi*-cephalomannine. In addition, the strong paclitaxel-like activities of these individual diastereomers, both in vitro and in vivo, were revealed by them. Despite this exciting progress, the stereochemical assignments at C2″ and C3″ of the dibrominated products of cephalomannine and its analogues have not yet been made, which became a major obstacle against further development of these potentially valuable bioactive compounds.



1	R_1=Bz, R_2=Ac, R_3=OH, R_4=H	**Paclitaxel**
2	R_1= Tigloyl, R_2=Ac, R_3=OH, R_4=H	**Cephalomannine**
3	R_1=Boc, R_2=H, R_3=OH, R_4=H	**Docetaxel**
4	R_1=Bz, R_2=H, R_3=H, R_4=OH	**7-*epi*-10-Deacetyltaxol**
5	R_1= Tigloyl, R_2=Ac, R_3=H, R_4=OH	**7-*epi*-10-Deacetylcephalomannine**

In this paper, the synthesis and evaluation of two dibrominated products (**6** and **7**) of 7-*epi*-10-deacetylcephalomannine (Scheme 1) are reported. More importantly, we report herein the crystal structure of **6**[†] and stereochemical assignments of **6** and **7** on the basis of an X-ray

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† CCDC 241319 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/data_request/cif, by emailing data_request@ccdc.cam.ac.uk, or by contacting The Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336033.

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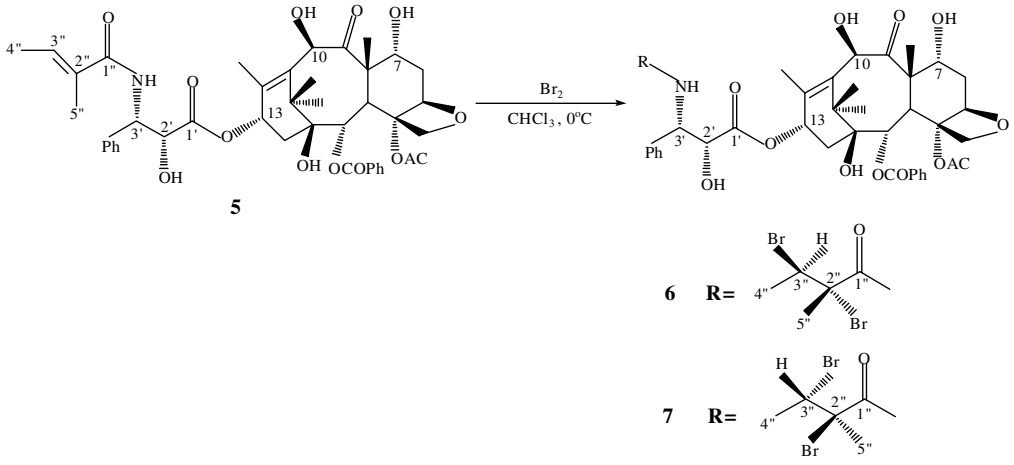

Scheme 1. Synthesis of compounds **6** and **7**.

single crystal diffraction study of **6**. The side-chain conformation of **6** is revealed to be a different one as compared to the two well-known conformations, that is, polar[7] and nonpolar[8] conformation found in the crystal structure of docetaxel,[9] 7-*epi*-10-deacetyltaxol[10] and paclitaxel,[11] respectively; however, most torsion angles of side chain of **6** were found to be very similar to those of the tubulin-bound T-shaped conformation.[12,13] The stereochemical assignments for **6** and **7** we report here can be reasonably used as a universal principle for stereochemical assignments of other dibrominated cephalomannine derivatives provided that similar elution systems to those reported here are used in the separation of these derivatives.

The diastereomers **6** and **7** were synthesized by reacting bromine with crude plant extract (3.0 g) containing 14.8% paclitaxel, 16.1% cephalomannine, 24.7% 7-*epi*-10-deacetylcephalomannine and 38.5% 7-*epi*-10-deacetylpaclitaxel in cooled chloroform. Isolation of the two diastereomers was performed by normal-phase and reverse-phase preparative chromatography in turn to obtain analytically pure samples (0.25 g **6** and 0.24 g **7**). Compound **6** eluted before compound **7** from a reverse-phase C_{18} column with water–acetonitrile (60:40) as the mobile phase. The two new brominated cephalomannine derivatives were characterized by NMR and APCI MS.[14]

Compared with the ^{1}H NMR spectrum of **5**,[15] two major differences in the ^{1}H NMR spectrum of **6** and **7** occur: (a) an upfield shift of the C3″ proton signal from ca. δ 6.43 to a quartet at ca. δ 4.6; (b) a downfield shift of the C5″ proton signal from a doublet at ca. δ 1.7 to singlets at ca. δ 1.99 and ca. δ 1.94, respectively. Other than the small difference in the C5″ proton signals, no obvious differences are observed between the ^{1}H NMR, ^{13}C NMR, H–H COSY and C–H COSY spectra of **6** and **7**, and we thus resort to X-ray crystallography to assign the stereochemistry of the adducts.

A colourless crystal of **6** of dimension $0.30 \times 0.25 \times 0.20$ mm^3 grown from CH_3CN was selected for diffraction studies. Molecular formula $C_{43}H_{51}Br_2NO_{13}$; molecular mass 949.67 amu; crystal system monoclinic; space group $C2$; unit cell dimensions $a = 20.692(6)$ Å; $b = 13.164(6)$ Å; $c = 20.479(7)$ Å; $V = 4815(3)$ Å^3; $D_{calcd} = 1.310$ mg/m^3; $F(0\,0\,0) = 1960$, $Z = 4$; Mo Kα = 0.71073 Å. Unit cell dimensions was determined by least-squares fit of 10,146 reflections in the range $1.15° < \theta < 25.01°$, which measured at 293(2) K using graphite monochromated Mo Kα radiations on a Bruker D8 Advance diffractometer. The total number of independent reflections measured was 7474. The structure was solved by direct methods and refined by full-matrix least-squares method on Fobs^2 by using the SHELXTL-97 software package. All non-H atoms were anisotropically refined. The hydrogen atoms were located by geometry calculation based on the related parent atoms. The final R and wR factors were 0.0789 and 0.2004, respectively. Attempts to obtain suitable crystals of **7** have so far been unsuccessful.

Figure 1 gives a perspective view of the molecular structure of **6** and atom labelling, which indicates that the stereochemistry at C2″ and C3″ can be unambiguously assigned as (2″*S*,3″*R*). Since bromination normally adopts an *anti*-addition mechanism,[16,17] the stereochemistry at C2″ and C3″ of **7** can be reasonably defined as (2″*R*,3″*S*).

As shown in Figure 1, the core tetracyclic ring system has a rigid structure. The cyclooctane ring (B) adopts the most stable boat–chair conformation, as shown by the torsion angles with atoms C1 and C9 as the ends. This ring is *trans*-fused along the C3–C8 bond to the six-membered ring C [atoms C7 and C4 deviated by −0.069 and 0.016 nm, respectively, from the mean plane of the other four atoms], which exhibits a chair conformation flattened in C4. Ring A, 'double-bridged' to the central ring, exhibits a boat conformation with C13 0.053 nm and C15 0.064 nm fully above the mean plane of the atoms C1, C11, C12 and C14. These conformations of the baccatin cores of **6** are more or less identical for all the baccatin derivatives resolved by X-ray analysis.[9–11,18,19]

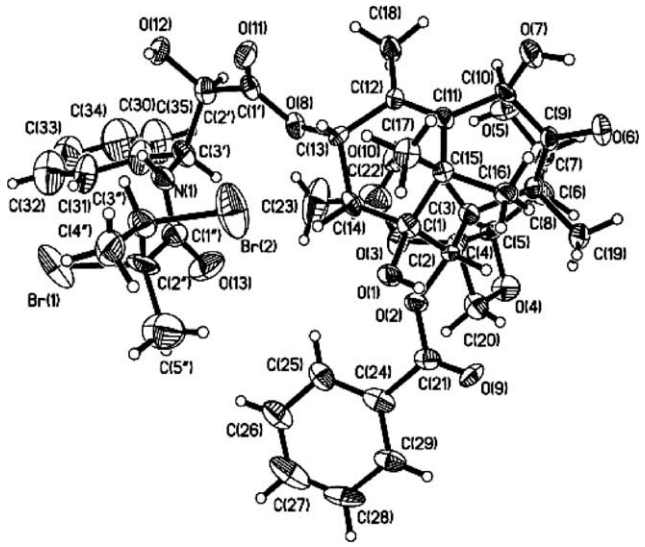

Figure 1. Molecular structure of **6**.

It is well established that the side-chain conformations of taxanes are related to their bioactivities and can be divided into three general types, that is, polar[7] and non-polar[8] and T-shaped conformations (T-Taxol).[12,13] Interestingly, the former two conformations have also been observed in crystal structures of paclitaxel[11] and its two active analogues, 7-*epi*-10-deacetyltaxol[10] and docetaxel.[9] The T-Taxol was recognized in the deconvolution of the average spectra of paclitaxel in chloroform[12] and proposed to be the bound conformation on β-tubulin.[13] Recent experimental evidence from highly active constrained taxol analogues proved that the T-Taxol conformation was very important to the tubulin-binding of Taxol.[20] The side chain at C13 in **6** is flexible and adopts a unique conformation. Table 1 lists the torsion angles of the C13 side chain for conformation of **6**, conformer A of 7-*epi*-10-deacetyltaxol, docetaxel and T-Taxol. Compound **6** assumes a similar geometry about the O8–C1′ bond as 7-*epi*-10-deacetyltaxol and about the C3′–N1 bond as both docetaxel and 7-*epi*-10-deacetyltaxol. The values of four torsion angles [C1′–C2′–C3′–N1, H2′–C2′–C3′–H3′, O12–C2′–C3′–N1, O12–C2′–C3′–C30] in **6** that involve in determining the geometry about C2′–C3′ bond are very similar to those in docetaxel, however, the value of torsion angle C1′–C2′–C3′–C30 of **6** and docetaxel contrast sharply, 178.3° and −179.4°, respectively. The 2′ and 3′ hydrogen atoms are *gauche* to each other as presented by the torsion angles H2′–C2′–C3′–H3′ = 56.5°, which is in good agreement with that of the crystal structure of docetaxel (57.2°) and cephalomannine in lipophilic solutions (52 ± 5°).[21] Compound **6** also adopts an essentially similar geometry about the N1–C1″ bond as docetaxel. The most dramatic difference between **6** and conformer A of 7-*epi*-10-deacetyltaxol and docetaxel occurs in the geometry about C1′–C2′ bond, two torsion angles [O8–C1′–C2′–C3′, O11–C1′–C2′–C3′] are very close to that of docetaxel but another two differ greatly with that of docetaxel and 7-*epi*-10-deacetyltaxol (A). To our surprise, most torsion angles of **6** and T-Taxol can be concluded as very similar with only one exception, [C3′–N1–C1″–C2″], which is −171.9° in **6** while 177.7° in T-Taxol. Difference on torsion angles of [C3′–N1–C1″–C2″] between T-Taxol and **6** corresponded to different substituted groups in the C3′–N position, which is a benzamidophenyl ring in T-Taxol whilst a dibrominated tigloyl moiety in **6**. In the crystal structure of **6**, four intramolecular hydrogen bonds involving the two bromine atoms have been observed (C4″–H4″3···Br1 = 110.97°, C4″···Br1 = 3.230 Å, H4″3···Br1 = 2.760 Å; C31–H31···Br1 = 159.00°, C31···Br1 = 3.832 Å, H31···Br1 = 2.949 Å; C14–H14B···Br2 = 134.88°, C14···Br2 = 3.687 Å, H14B···Br2 = 2.937 Å; C5″–H5″A···Br2 = 110.84°, C5″···Br2 = 3.305 Å, H5″A···Br2 = 2.840 Å). It appears that these hydrogen bonds are at least in part responsible for the unique side-chain conformation of **6**.

The in vitro biological activities of paclitaxel (**1**), **6** and **7** were evaluated in three cell lines including MCF-7 (breast carcinoma), A2780 (ovarian carcinoma) and A549 (mice carcinoma) cell lines (Table 2). The cell

Table 1. Selected torsion angles (°) for the side chain of **6**, conformer A of 7-*epi*-10-deacetyltaxol, docetaxel and T-Taxol

	6	7-*epi*-10-Deacetyltaxol (A)	Docetaxel	T-Taxol
C13–O8–C1′–O11	8.9	11.8	−6.6	0.6
C13–O8–C1′–C2′	−169.4	−166.6	168.0	−178.7
O8–C1′–C2′–O12	179.3	−134.0	−176.7	178.1
O8–C1′–C2′–C3′	56.2	108.3	60.2	55.7
O11–C1′–C2′–O12	1.0	47.5	−2.2	−1.2
O11–C1′–C2′–C3′	−122.1	−70.0	−125.3	−123.7
C1′–C2′–C3′–C30	178.3	−66.0	−179.4	178.0
C1′–C2′–C3′–N1	53.4	168.8	56.4	52.4
H2′–C2′–C3′–H3′	56.5	64.2	57.3	55.4
O12–C2′–C3′–N1	−68.7	51.7	−64.7	−67.0
O12–C2′–C3′–C30	56.2	176.4	59.5	58.5
C2′–C3′–N1–C1″	−138.0	−143.0	−141.3	−153.4
H3′–C3′–N1–H1A(N1)	157.7	153.6	159.4	147.3
C30–C3′–N1–C1″	95.9	92.4	97.3	82.8
C3′–N1–C1″–O13	6.1	−3.0	12.8	−1.0
C3′–N1–C1″–C2″[a]	−171.9	177.0	−172.4	177.7

[a] In docetaxel,[9] the corresponding torsion angle is C3′–N10′–C11′–O12′.

Table 2. Cytotoxicities of paclitaxel and new brominated analogues in three tumour cell lines (IC_{50}[a], μg/mL)

	MCF-7	A549	A2780
Paclitaxel	1.25×10^{-3}	2.0×10^{-3}	6.76×10^{-3}
6	7.10×10^{-3}	2.69×10^{-2}	4.84×10^{-3}
7	4.75×10^{-3}	1.30×10^{-2}	2.13×10^{-3}

[a] IC_{50} (μg/mL), concentration required to inhibit cell growth by 50% on continuous exposure time of 72 h.

concentration was 1000 cells/well. All drugs were tested at an incubation time of 72 h and then analyzed for cytotoxic activity using the MTT procedure. As shown in Table 2, as to A2780 and MCF-7, **6** and **7** have the cytotoxicity comparable to paclitaxel, while the cytotoxicity of paclitaxel against A549 is about 10 times stronger than those of **6** and **7**.

In conclusion we have synthesized and purified two diastereomers of 2″,3″-dibrom-7-*epi*-10-deacetylcephalomannine, which show strong in vitro activity. The stereochemistry at C2″ and C3″ is determined by means of crystallographic study of **6** and the side-chain conformation of **6** is different with the well-known polar and apolar conformation. Most torsion angles of C13 side chain of **6** and T-Taxol are very similar. Such an unexpected and important result make it of great significance to study further the solution conformation and binding conformation of **6** by using NAMFIS method (*N*MR *a*nalysis of *m*olecular *f*lexibility *i*n *s*olution)[22] and Dock methodology,[23] respectively, which is now underway in collaboration with others. The final results will be published elsewhere in the near future.

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14. Spectral data for compounds **6** and **7**. *Compound* **6**: mp 171–173 °C, UV λ_{max} MeOH (ε) 195 nm; ^{1}H NMR ($CDCl_3$): δ 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 = 2.27, 2.35, 1H), 3.91 (d, J = 7.37, 1H), 4.39 (t, J = 9.39, 2H), 4.60 (q, 1H), 4.71 (d, 1H), 4.90 (dd, J = 3.92, 3.76, 1H), 5.42 (s, 1H), 5.56 (dd, J = 1.76, 1.95, 1H), 5.73 (d, J = 7.37, 1H), 6.22 (t, J = 8.45, 1H), 7.36–8.12 (m, 11H); ^{13}C NMR ($CDCl_3$): δ 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; APCI full MS *m*/*z*: [M+Na$^+$] 971.58. *Compound* **7**: mp 176–178 °C, UV λ_{max} MeOH (ε) 196 nm; ^{1}H NMR ($CDCl_3$): δ 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 = 2.18, 2.60, 1H), 3.91 (d, J = 7.30, 1H), 4.39 (t, J = 8.93, 2H), 4.60 (q, 1H), 4.72 (d, 1H), 4.89 (dd, J = 3.81, 3.76, 1H) 5.43 (s, 1H), 5.58 (dd, J = 1.79, 1.96, 1H), 5.72 (d, J = 7.30, 1H), 6.24 (t, J = 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; APCI full MS *m*/*z*: [M+Na]$^+$ 972.18.
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