Synthesis and Separation of Potential Anticancer Active Dihalocephalomannine Diastereomers from Extracts of *Taxus yunnanensis*

Ramesh C. Pandey,* Luben K. Yankov, Alexander Poulev, Raghu Nair, and Salvatore Caccamese†

Xechem, Inc., Research Laboratories, 100 Jersey Avenue, Building B-310, New Brunswick, New Jersey 08901-3279

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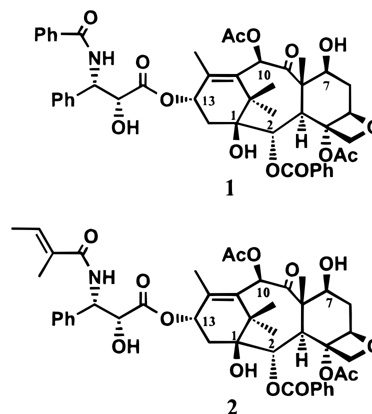
Two diastereomeric 2″,3″-dibromo cephalomannines and their two corresponding 7-epimers were obtained by treatment of extracts of *Taxus yunnanensis* with bromine solution under mild conditions. Treatment of the same extract with chlorine solution yielded four diasteromeric 2″,3″-dichlorocephalomannines, with no 7-epimers. The diastereomeric mixtures were separated into the individual components by preparative HPLC on C_{18} reversed-phase silica gel. A more efficient analytical separation was obtained on a pentafluorophenyl-bonded phase. These reaction products were isolated and fully identified spectroscopically. Slight differences were observed in the NMR spectra of the 7-epimers when compared to their 7β-OH analogues. On the basis of a comparison of physicochemical data, the bromo compounds were identified as (2″*R*,3″*S*) -dibromo-7-*epi*-cephalomannine **(3)**, (2″*S*,3″*R*)-dibromo-7-*epi*-cephalomannine **(4)**, (2″*R*,3″*S*)-dibromocephalomannine **(5)**, and (2″*S*,3″*R*)-dibromocephalomannine **(6),** and the chloro compounds as (2″*R*,3″*R*)-dichlorocephalomannine **(7)**, (2″*S*,3″*S*)-dichlorocephalomannine **(8)**, (2″*R*,3″*S*)-dichlorocephalomannine **(9)**, and (2″*S*,3″*R*)-dichlorocephalomannine **(10)**. Cytotoxic activity was tested against the NCI 60 human tumor cell line panel in comparison with paclitaxel (**1**), and encouraging results were obtained in some cases.

The diterpenoid paclitaxel (**1**) has been added in recent years into the armamentarium of clinical therapeutic agents against certain human solid tumors, such as ovarian and breast cancers, and is currently in clinical trial for the treatment of lung, colon, and other cancers.[1,2] The isolation of paclitaxel from *Taxus brevifolia* bark (Pacific yew tree)[3−5] and other *Taxus* species including the ornamental yew, *T. baccata*,[6,7] as well as *T. yunnanensis*,[8] and *T. cuspidata*[9,10] is a challenging task due to the very low concentration (0.002−0.04% of the extracted dry weight) of **1** and to the fact that paclitaxel occurs with the structurally related cephalomannine (**2**) and a series of other taxanes. For example, a recent HPLC analysis of the taxane fraction from ornamental *Taxus* needles indicated nine identified taxanes,[11] and a review has mentioned the presence, beside known compounds, of 24 additional taxanes as very minor components of *T. baccata* and *T. wallichiana.*[12]

The HPLC separation of a crude extract or a mixture of **1** and **2** requires exacting conditions and is an expensive step in a large-scale commercial preparation of paclitaxel. Methods other than chromatographic separation of paclitaxel from cephalomannine have been developed, such as chemical modification of the side-chain double bond in cephalomannine. Kingston et al. reacted a mixture of **1** and **2** with OsO_4, and a selective formation of a diastereomeric mixture of diols in the side-chain double bond of **2** occurred.[13] Paclitaxel (**1**) was subsequently obtained in good yield, but the diastereomeric diols were not separated.[13] Murray et al. described the selective oxidation of the side-chain double bond of **2** by ozone and subsequent preparation of water-soluble hydrazones easily separable from **1**.[14,15] Recently, Rimoldi et al. reported the treatment of a mixture of **1** and **2** with bromine in $CHCl_3$ at room temperature for 5 min, which yielded unchanged paclitaxel **(1)** and a mixture of dibrominated compounds.[16] Paclitaxel was subsequently isolated but the diastereomeric dibromides were not separated.[16]



In this paper, we report convenient methods for the selective bromination and chlorination of the side-chain double bond of **2** to produce a diastereomeric mixture of dihalocephalomannines (**3**−**10**) and the separation of the individual diastereomers.[17,18] More specifically, we report the large-scale development of bromination and chlorination processes employing experimental conditions very different from those reported by Rimoldi *et al.*[16] and that utilize as the starting product a taxane-enriched fraction of an extract of *Taxus yunnanensis*. Further, an HPLC method, which uses a pentafluorophenyl phase-bonded column, was developed to separate the diastereomers, which were then fully characterized. The isolated and purified mixture of the

* To whom correspondence should be addressed. Phone: (732) 247-3300. Fax: (732) 247-4090.

† Visiting Professor from Dipartimento di Scienze Chimiche, University of Catania, 95125 Catania, Italy.

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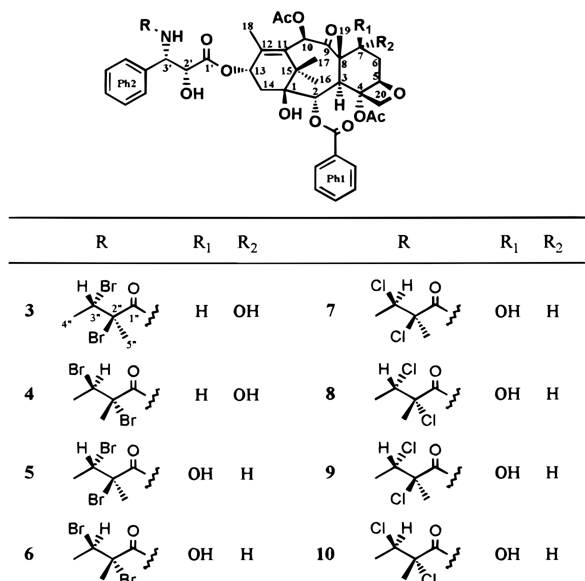

	R	R_1	R_2
3		H	OH
4		H	OH
5		OH	H
6		OH	H
7		OH	H
8		OH	H
9		OH	H
10		OH	H

dihalocephalomannines exhibited strong paclitaxel-like in vitro cytotoxic activity. In the method reported herein, cephalomannine (**2**), a close analogue of paclitaxel (**1**), which in the past has interfered in the purification process of paclitaxel, was converted into a group of potentially valuable bioactive compounds. In addition to the separation of the dihalogenated compounds, a convenient isolation and purification procedure for pure paclitaxel is reported.

Results and Discussion

A taxane-containing fraction from a crude extract of *T. yunnanensis* was subjected to a bromination reaction. This reaction can be applied to taxane fractions containing more than 50% of paclitaxel (**1**) and less than 30% of cephalomannine (**2**) from other *Taxus* species.[17] To a very dilute (2 g/L) cooled (0−5 °C) solution in CCl_4−CH_2Cl_2 was added a solution of bromine (0.1 M in CCl_4) dropwise and under stirring in the dark so that the temperature remained below 5 °C. The progress of the reaction was monitored by HPLC, observing the area ratio of the paclitaxel/cephalomannine peaks. In Figure 1, typical HPLC chromatograms of an extract before and after bromination are given (a and b, respectively). The identities of the peaks were assigned by spiking with standard compounds and by measuring the UV spectrum of the eluting peaks with a photodiode array detector. At the end of the reaction, the cephalomannine (**2**) peak almost disappeared, while the peak corresponding to paclitaxel (**1**) remained almost unchanged. Broad peaks (retention times ca. 35 and 42 min) corresponded to diastereomeric mixtures of **5**/**6** and **3**/**4**, respectively.

After workup to eliminate excess bromine (see the Experimental Section), the solid residue was subjected to MPLC on silica gel, eluting with 1,2-dichloroethane containing 1.5% methanol. The C_{18} reversed-phase HPLC separation (CH_3CN−H_2O, 1:1) of low-polarity selected fractions yielded a mixture of the dibromo-7-*epi*-cephalomannines **3** and **4**, while midpolarity-selected fractions afforded a mixture of the dibromocephalomannines **5** and **6**. Figure 1c,d shows typical chromatograms of a fraction containing the pair **3**/**4** and of a

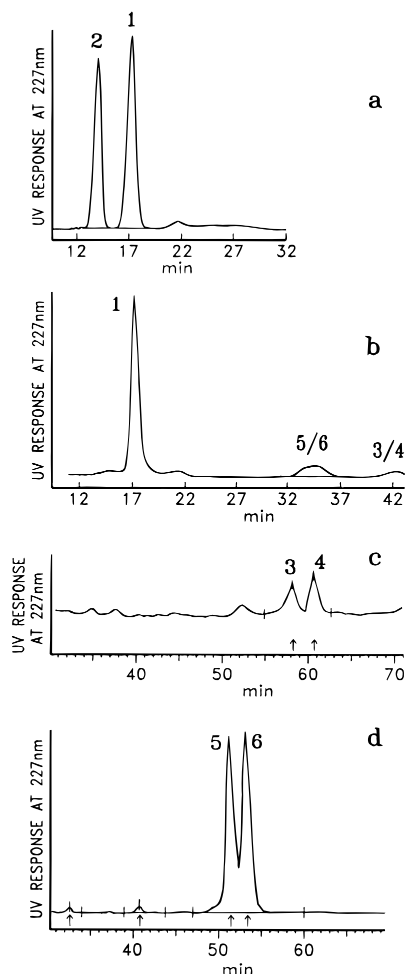

Figure 1. HPLC chromatograms of (a) the taxane fraction of *Taxus yunnanensis* before bromination and (b) after bromination, (c) a chromatographic fraction containing the pair **3**/**4**, and (d) a chromatographic fraction containing the pair **5**/**6**. Conditions: (a) and (b) phenyl column with CH_3CN−H_2O−CH_3OH (32:48:20) at 1 mL/min; (c) and (d) octadecylsilyl (ODS) column with CH_3CN−H_2O−CH_3OH (45:40:15) at 0.5 mL/min.

fraction containing the pair **5**/**6**, respectively. A clear preliminary clue that a mixture of compounds **5** and **6** was present came from differential scanning calorimetry (DSC) that showed two distinct peaks at 173.76 and 187.73 °C, under heating in nitrogen at 5 °C/min.

Resolution of the diastereomeric pairs was a challenging task. Indeed, several combinations of stationary and mobile phases were tried before it was possible to optimize the conditions. As an example, the conditions shown in Figure 1a,b did not give any separation of the components of the diastereomeric mixture, while the conditions shown in Figure 1c,d afforded a good separation of the individual components of the mixture, with a separation factor α^{19} of 1.04 and 1.05 being measured for the pair **3**/**4** and pair **5**/**6**, respectively. From the highest polarity selected fractions of the MPLC, by evaporation and crystallization, paclitaxel (**1**) was isolated and identified by comparison of its physical and spectral data, including optical rotation. The mild reaction conditions (dilution, low temperature, and solvent) afforded a selective bromination of the side-chain double bond in cephalomannine (**2**) and its 7-epimer. The presence of 2″,3″-dibromocephalomannines in the reaction mixture was confirmed by a

Table 1. Selected ^{1}H NMR Data of Compounds **3**–**6** ($CDCl_3$, 300 MHz)

proton	**3**[a]	5[b]
3	3.90 (d, 7.0)	3.79 (d, 7.0)
6α	2.32 (m)	2.35 (m)
6β	2.25 (m)	1.80 (m)
7	3.72 (m)	4.41 (m)
7-OH	4.62 (m)	2.46 (b s)
10	6.79 (s)	6.28 (s)
14	2.05–2.40 (m)	2.30 (m)
20α	4.38 (m)	4.29 (d, 8.4)
20β	4.38 (m)	4.20 (d, 8.4)
2′	4.74 (b d, 4.9)	4.74 (b d, 4.9)
2′-OH	3.23 (br s)	3.36 (br s)
3″	4.61 (q, 6.6)	4.61 (q, 6.6)
4″	1.70 (d, 6.6)	1.80 (d, 6.6)
5″	1.98 (s)	2.00 (s)
Ph2	7.30–7.70 (m)	7.30–7.70 (m)

[a] Compound **4** exhibited identical values except for H-5″: δ 1.95 (s). [b] Compound **6** exhibited identical values except for OH-2′: δ 3.45 (br s).

characteristic dibrominated $[M + CH_3CN]^+$ ion at *m*/*z* 1032 and by the $[M + Na]^+$ ion in the APCI HPLC/MS experiment. The MS of other eluting peaks of the reaction mixture did not show fragment ions resulting from the addition or substitution of bromine into the taxane ring. Indeed, more concentrated solutions of the crude extracts and room-temperature reaction gave a more complex HPLC profile of the reaction mixture.

Sterically hindered double bonds, as in the case of the Δ^{11} in the taxane ring, are known to undergo electrophilic substitution in halogenated solvents and at room temperature to give a complex mixture of brominated compounds.[20] The presence of the C-7 epimers of cephalomannine can be artifactual from the chromatographic purification of the reaction mixture on silica gel or during bromination.[21]

The positive-ion FABMS of the product **5** revealed a dibrominated $[M+H]^+$ peak at *m*/*z* 992.189 05, corresponding to the composition $C_{45}H_{54}NO_{14}{}^{79}Br^{81}Br$. The presence of the intact taxane moiety was suggested by the peaks at *m*/*z* 569 (corresponding to the taxane ring $[T]^+$) and *m*/*z* 509 $[T - AcOH]^+$ and by the presence of dibrominated side chain [SBr] "complementary" peaks at *m*/*z* 424 $[SBr + 2H]^+$, *m*/*z* 404 $[S - H_2O]^+$, and the very intense peak at *m*/*z* 376 $[SBr - CO_2 - 2H]^+$. All fragments of the [SBr] series possessed the isotopic pattern typical of dibrominated compounds. Diastereomers **3**, **4**, and **6** exhibited the same fragmentation pattern, and the relative abundances of the abovementioned peaks were almost equal. The selected ^{1}H NMR resonances of compounds **5** and **6** are listed in Table 1. Rimoldi et al. reported and assigned only some resonances in the ^{1}H NMR spectrum of the diastereomeric mixture.[16] In the ^{13}C NMR spectra of **5** and **6** (Table 2), reported here for the first time, the chemical shifts of C-2″ and C-3″ were shifted by several ppm in comparison with cephalomannine (**2**)[22,23] due to the new character of the halogenated sp^3 carbons. A slight shift in the signal for C-1″ was attributed to the new vicinal brominated C-2″. The signals for the C-11 and C-12 sp^2 carbons remained unchanged. From the above data, the structures of (2″*R*,3″*S*)- and (2″*S*,3″*R*)-dibromocephalomannine, respectively, could be assigned to compounds **5** and **6**. Indeed, we assumed that the usual stereoselective *anti*-addition of halogen at the double bond took place. At this stage, the stereochemistry at carbons 2″ and 3″ can be interchanged between **5** and **6**. No significant differences were in fact observed in the ^{1}H and ^{13}C NMR spectra of these compounds. Significant differences occurred in their UV, HPLC, TLC, and mp properties, which were not useful for stereochemical assignments.

Table 2. Selected ^{13}C NMR Data of Compounds **3**–**6** ($CDCl_3$, 75 MHz)

carbon	**3**	**4**	**5**	**6**
7	75.8	75.9	72.3	72.2
9	207.1	207.1	203.5	203.5
11	133.8	133.7	133.8[a]	133.7[a]
12	139.6	139.6	141.8	141.8
19	16.2	16.2	9.6	9.6
1′	172.5	172.5	172.4	172.4
2	72.9	72.9	73.0	72.9
3′	54.0	54.1	54.6	54.6
10-OAc C=O	169.3	169.3	170.3	170.3
10-OAc Me	20.8	20.8	20.8	20.8
i-Ph1	128.5[a]	128.5[a]	128.5[b]	128.5[b]
o-Ph1	130.3	130.4	130.3	130.3
m-Ph1	128.8[a]	128.8[a]	128.8[b]	128.8[b]
p-Ph1	137.5	137.4	137.5	137.4
i-Ph2	129.5	129.6	129.3	129.4
o-Ph2	126.7	126.8	126.9	126.9
m-Ph2	129.1[a]	129.1[a]	128.9[b]	129.0[b]
p-Ph2	131.5	131.6	133.6[a]	133.6[a]
1″	168.8	168.9	168.7	168.8
2″	57.7	57.8	58.8	58.8
3″	54.3	54.3	55.4	55.2
4″	22.6	22.6	22.7	22.7
5″	27.2	27.6	27.6	27.9

[a,b] Signals with the same superscript may be interchanged.

The ^{1}H and ^{13}C NMR spectra, in comparison with the literature data, were helpful in assigning a 7-epimeric structure to the diastereomeric compounds **3** and **4**. The structural relationship between 7-*epi*-cephalomannine and **3**/**4** arose from the comparison of their spectra (Tables 1 and 2). The major differences between the ^{1}H NMR spectra of pairs **3**/**4** and **5**/**6** were the downfield shifts of H-3, H-10, and H_2-20. Dramatic were the changes in the chemical shifts of H-7, 7-OH, H-6β, H-14, and 2′-OH and were consistent with epimerization at C-7, as reported in 7-*epi*-paclitaxel[22,24] and 7-*epi*-cephalomannine.[23] In the ^{13}C NMR spectrum of **3**/**4**, the chemical shifts of C-7, C-9, and C-19 were different with respect to those of **5**/**6** and again supported a 7α-orientation of the hydroxyl groups. The UV spectra of **3**/**4** both showed a small but significant hypsochromic shift at the peak maximum (219 nm). The IR spectra of **3**–**6** were almost identical. Also in this case an interchangeable assignment of the stereochemistry of C-2″ and 3″ in compounds **3** and **4** is possible.

We also carried out a chlorination reaction, hitherto unreported, to modify the side-chain double bond of cephalomannine (**2**). The taxane fraction of the same extract, used for bromination, was dissolved at 4 °C in 1,2-dichloroethane containing an excess of chlorine and reacted for 3 h, following the disappearance of the cephalomannine peak by reversed-phase HPLC. At this time, the reaction was quenched with ice–water. After workup of the reaction mixture (see the Experimental Section), a solid residue was obtained. From this, paclitaxel (**1**) was separated by repeated crystallizations using acetone–hexane mixtures. Since chromatographic separation of **1** from the chlorination products was not necessary, this procedure offers a convenient

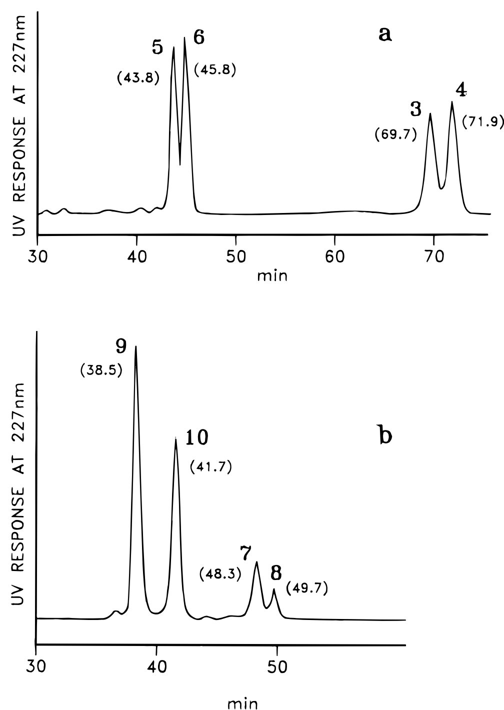

Figure 2. HPLC chromatograms of (a) a mixture of compounds **3**−**6** and (b) a mixture of compounds **7**−**10.** Conditions: pentafluorophenyl column with $H_2O-CH_3CN-CH_3OH$ (41:39:20) at 0.5 mL/min.

alternative to the isolation of **1** as a production process. Also in this case, appropriate dilution of the taxane fraction submitted to chlorination (3.2 g/L) and temperature were crucial to avoid secondary reactions. The mother liquors from the crystallizations of **1** were combined and the diastereomers separated by preparative C_{18} reversed-phase HPLC (CH_3CN-H_2O) yielding dichlorocephalomannine mixtures **7/8** from the low-polarity fractions and **9/10** from the midpolarity fractions. For analytical purposes, a dedicated and more efficient column (pentafluorophenyl reversed-phase) gave better separation factors and much shorter elution times when compared to the C_{18} phase. The HPLC chromatograms of all compounds (**3**−**10**) obtained with this stationary phase are reported in Figure 2. It is interesting to note the separation in the HPLC (Figure 2a) of the two dibromocephalomannines from the two dibromo-7-*epi*-cephalomannines, when compared with the four chloro derivatives (Figure 2b). The separation factor α was better for the pair **9/10** (1.09) than for the other pairs **3/4**, **5/6**, and **7/8** (1.05). Under identical HPLC conditions, the separation factors for **1/2** and **1/9** were 1.15 and 1.71, respectively. This again showed a more convenient separation of **1** from the mixture of dichlorocephalomannines (**9/10**) compared to the separation of **1** from **2**.

The FABMS of product **9** revealed a $[M + H]^+$ peak at m/z 902 corresponding to the elemental composition $C_{45}H_{54}NO_{14}{}^{35}Cl_2$. Analogous to compound **5**, the presence of the intact taxane moiety was confirmed by the peaks at m/z 569 and 509 representing the taxane ring $[T]^+$ and $[T - AcOH]^+$, respectively. The presence of a dichlorinated side chain [SCl] was confirmed by the peaks at m/z 333 $[SCl + H]^+$ and 288 $[SCl - CO_2]^+$. All fragments of the [SCl] series possess the isotopic pattern typical of dichloro compounds. Diastereomers **7**, **8**, and

Table 3. Selected ^1H NMR Data of Compounds **7**−**10** ($CDCl_3$, 300 MHz)

proton	**7**[a]	**9**[b]
3	3.82 (br d)	3.80 (d, 7.0)
4-OAc	2.40 (s)	2.38 (s)
6α	2.54 (m)	2.54 (m)
6β	1.90 (m)	1.90 (m)
7	4.40 (m)	4.41 (m)
7-OH	2.53 (m)	2.54 (m)
10	6.26 (s)	6.31 (s)
14	2.32 (m)	2.45 (m)
20α	4.35 (d, 8.4)	4.32 (d, 8.4)
20β	4.15 (d, 8.4)	4.20 (d, 8.4)
2′	4.70 (br d, 5)	4.72 (br d, 5)
2′-OH	3.36 (br s)	3.36 (b s)
3″	4.50 (q, 6.6)	4.58 (q, 6.6)
4″	1.48 (d, 6.6)	1.55 (d, 6.6)
5″	1.50 (s)	1.59 (s)
Ph2	7.35−7.68 (m)	7.40−7.68 (m)

[a] Compound **8** exhibited identical values except for OH-2′: δ 3.31 (br s). [b] Compound **10** exhibited identical values except for H-6α: δ 2.56 (m); OH-2′: δ 3.45 (br s).

Table 4. Selected ^{13}C NMR Data of Compounds **7**−**10** ($CDCl_3$, 75 MHz)

carbon	**7**	**8**	**9**	**10**
7	72.2	72.2	72.1	72.1
9	203.5	203.5	203.5	203.5
11	133.8	133.8	133.6[a]	133.7[a]
12	141.8	141.8	142.0	141.9
19	9.6	9.6	9.5	9.5
1′	172.2	172.2	172.0	172.6
2′	73.0	72.9	73.1	72.6
3′	54.8	54.8	55.0	55.0
10-OAc C=O	170.2	170.3	170.2	170.2
10-OAc Me	20.9	20.8	19.1	19.1
i-Ph1	128.6[a]	128.6[a]	128.3[b]	128.3[b]
o-Ph1	130.2	130.2	130.2	130.2
m-Ph1	128.7[a]	128.7[i]	128.7[b]	128.7[b]
p-Ph1	137.4	137.4	137.8	136.9
i-Ph2	129.2	129.2	129.4	129.2
o-Ph2	126.8	126.9	126.7	126.9
m-Ph2	129.1	129.1	129.0	128.9
p-Ph2	133.4	133.4	133.3[a]	133.3[a]
1″	169.3	169.4	169.3	169.9
2″	62.7	62.5	61.4	61.3
3″	55.3	55.0	58.7	58.7
4″	21.6	21.7	21.8	21.8
5″	29.3	29.3	27.5	27.7

[a,b] Signals with the same superscript may be interchanged.

10 had the same fragmentation pattern, and the relative abundances of the above-mentioned peaks were almost equal. The IR spectra of **7**−**10** were almost identical. In the ^1H NMR spectrum of **9** (Table 3), the signal at 6.31 ppm attributed to the acidic H-10 was almost coincident with the analogous data for compound **2**,[23] which demonstrated that no chlorine substitution occurred at that position. The observation of the characteristic H_2-20 AB system in **7**−**10** confirmed an intact oxetane ring and ruled out 7-epimerization.[22] As for compound **5**, the ^{13}C NMR spectra of **9** and **10** (Table 4) showed the modification at the side chain with respect to **2**, while no changes occurred in the taxane ring resonances. There were no differences in the ^{13}C NMR assignments for C-7, C-9, and C-19 in **7**−**10**, and this again excludes epimeric structures.[22] Also, in this case, the configuration 2″*R*,3″*S* assigned to compound **9** can be interchanged with the configuration 2″*S*,3″*R* assigned to compound **10**. Compounds **7** and **8** could then be assigned as (2″*R*,3″*R*)-dichlorocephalomannine **(7)** and (2″*S*,3″*S*)-dichlorocephalomannine **(8),** respectively.

Table 5. Comparison of in Vitro Cytotoxicity Potencies of Paclitaxel (**1**) and Compounds **5/6**, **9**, and **10**[a]

compound	GI_{50}[b]	TGI[b]	LC_{50}[b]
1	6.5×10^{-10}	1.5×10^{-7}	6.2×10^{-7}
5/6	3.6×10^{-8}	3.5×10^{-6}	3.8×10^{-5}
9	2.1×10^{-7}	6.3×10^{-6}	3.2×10^{-5}
10	1.4×10^{-7}	4.2×10^{-6}	2.9×10^{-5}

[a] Evaluated in the NCI 60-cell-line human tumor screening panel; data reported (molarity) are overall panel averages (MG-MID, according to ref 25). [b] GI_{50} drug-treated cell net growth is 50% of untreated controls; TGI, no net growth of drug-treated cells; LC_{50}, 50% net cell death in drug-treated cells vs starting levels.

The configurations of **7** and **8** can be interchanged. These stereochemical assignments are consistent with the relative amount of **9/10** and **7/8** pairs obtained in the chlorination. In fact, the first pair **9/10** is obtained by the usual *anti*-addition of chlorine at the double bond, while the second pair **7/8** involves, instead, a more difficult *syn*-addition. It is remarkable that no epimerization occurred during chlorination, contrary to bromination.

Table 5 shows the results of comparative evaluation of paclitaxel (**1**), the dibrominated mixture **5/6**, and compounds **9** and **10** in the NCI screen of 60 human tumor cell lines.[25] The pattern of differential cytotoxicity for mixture **5/6** was slightly better than that for compounds **9** and **10** and showed a slight reduction with respect to **1**, with an average TGI value of 3.5×10^{-6} M compared to 1.5×10^{-7} M for **1**. Nevertheless, the values for these dihalogenated compounds (Table 5) indicate an unusually high degree of potency. The dibrominated mixture **5/6** exhibited a promising response against COLO 205 (colon), OVCAR 3 (ovarian), and MCF-7 (breast) cancer cell lines included in the screening panel, with the TGI value being superior to that of paclitaxel (**1**). Further details on these and other experiments on human tumor cells will be reported elsewhere (Pandey, R. C.; Yankov, L. K. Unpublished data).

In conclusion, we have provided a large-scale process for the production of dihalocephalomannines from cephalomannine (**2**), a side product in the paclitaxel **(1)** isolation, via a selective halogenation of **2** under mild conditions, starting from extracts of raw *T. yunnanensis* biomass. In addition, we have separated efficiently the diastereomeric products resulting from the reaction using a bonded reversed-phase column. Paclitaxel was also isolated from the dichlorinated reaction mixture by a simple method that did not require chromatography. The diastereomeric halogenated products exhibited encouraging cytotoxic activity and will be submitted to other diverse biological screens.

Experimental Section

General Experimental Procedures. Melting points were determined with a Bristolscope hot-stage microscope and are uncorrected. Optical rotations were measured with a Rudolph Digipol 1 A31 polarimeter. Differential scanning calorimetry (DSC) was recorded on a DuPont Thermal Analyst 2000 Instrument at Detroit Testing Laboratory, Warren, MI. IR and UV spectra were obtained using a Perkin-Elmer 683 and a Perkin-Elmer Lamba 5 spectrophotometer, respectively. NMR spectra were recorded on a Bruker AM 300 spectrometer, with TMS as the internal standard. The FABMS experiments were performed with a VG ZAB-T mass spectrometer, using dithiothreitol−dithioerythritol (3:1) in MeOH as matrix.

Analytical HPLC was carried out on Waters model 600 E equipped with 712 WISP and 990 photodiode array detector and a Waters model 510 equipped with a 490 programmable multiwavelength detector. Preparative HPLC was performed on a Waters DeltaPrep 3000 instrument. Medium-pressure liquid chromatography (MPLC) was performed on a Büchi B-680 A instrument (Büchi Laboratoriums, Flawil, Switzerland) using a Büchi glass column filled with silica gel 60 (40−63 μm) from EM Science (Gibbstone, NJ). The analytical columns (4.6 mm × 250 mm) used were pentafluorophenyl (trade name FSP) from ES Industries (Marlton, NJ), Spherisorb octadecylsilyl (ODS 5) from Phenomenex (Torrance, CA), and μBondapak phenyl from Waters (Milford, MA). A Deltapak C_{18} column (19 mm × 300 mm) from Waters was used for preparative HPLC. LC/MS analysis was performed using a Fisons VG Platform II instrument equipped with a VG Mass Lynx data system and interfaced with a Varian 9012 HPLC utilizing a Curosil 6 (Phenomenex) column with the mobile phase, 0.01 M NH_4OAc−MeOH−CH_3CN (60:5:35), at a flow rate 0.9 mL/min. TLC was conducted on ready-made silica gel 60 F_{254} plates from E. Merck (Darmstadt, Germany). Detection was carried out by spraying with 0.1% vanillin in concentrated H_2SO_4 in methanol (1:1) and subsequent heating at 105 °C for 3 min. The bromine solution was 0.1 M in CCl_4 from Baker. The chlorine solution was prepared by slow bubbling of chlorine gas (Aldrich) into precooled 1,2-dichloroethane. The dissolved chlorine content was determined by standard $KI/Na_2S_2O_3$ titration after acidification with HCl.

Plant Material. Extracts of *T. yunnanensis* Cheng (Taxaceae) were obtained from Guizhou Fanya Pharmaceuticals Co., Guiyang, People's Republic of China, after extraction of the bark with ethanol, partitioning of the dried extract with $CHCl_3$−H_2O, and subsequent silica gel flash chromatography of the $CHCl_3$ extract. Aliquots of this extract (containing 28% cephalomannine (**2**), 60% paclitaxel (**1**), and other taxanes) were used in the halogenation experiments.

Bromination Reaction. To a stirred solution (cooled in an ice bath and kept in the dark) of 10 g of the crude extract of *T. yunnanensis,* in 5 L of CCl_4−CH_2Cl_2 (4.8:0.2), was added dropwise 40 mL of 0.1 M bromine in CCl_4. An additional 7 mL of bromine solution were added after 7, 14, and 21 h. At the end of the reaction the molar ratio bromine/cephalomannine was 1.8. The reaction progress was monitored by HPLC using a μBondapak phenyl column. The reaction mixture was quenched with 5 L of cooled 0.2% Na_2SO_3 solution and washed with water (2 × 5 L). The aqueous phase was extracted with CH_2Cl_2 (2 × 500 mL). The combined organic layers were dried (Na_2SO_4) and evaporated under reduced pressure to obtain 13.6 g of a solid mixture. This was dissolved in 1,2-dichloroethane and applied to MPLC using silica gel (6.9 × 70 cm) as adsorbent and eluted with 1,2-dichloroethane−MeOH (98.5:1.5). Fractions of 500 mL were collected, and those exhibiting similar TLC profiles combined. The dibromo-7-*epi*-cephalomannines **3/4** were eluted in fractions 10−

14 (1.42 g), and the dibromocephalomannines **5**/**6** were eluted in fractions 24−28 (1.64 g). Paclitaxel (**1**) was eluted in fractions 34−54 (4.79 g) and identified by comparison of its physical and spectral data with those previously reported.[3,22]

Separation of Dibromo-7-*epi*-cephalomannines 3 and 4 and Dibromocephalomannines 5 and 6 by HPLC. The final purification of compounds **3**−**6** was done by means of preparative HPLC on a Waters Deltapak C_{18} column (19 mm × 30 cm) with CH_3CN−H_2O (1:1) at 15 mL/min as mobile phase. Portions (200 mg) of the mixture **3**−**6** dissolved in MeOH (2 mL) were injected. Elution times were approximately 54 (**5**), 56 (**6**), 104 (**3**), and 112 min (**4**). Seven consecutive injections, with the collection of the eluents and evaporation under reduced pressure of the solvent, yielded compounds **3** (14 mg), **4** (17 mg), **5** (185 mg), and **6** (182 mg) as white-pale yellow crystals. For analytical HPLC, 4.6 × 250 mm, 5 μm columns were used as follows: condition 1, pentafluorophenyl column with H_2O−MeCN−MeOH (41:39:20) at 0.5 mL/min as mobile phase; condition 2, octadecylsilyl column with H_2O−MeCN−MeOH (45:40:15) at 0.5 mL/min as mobile phase.

(2″*R*,3″*S*)-Dibromo-7-*epi*-cephalomannine (3): mp 166−168 °C, $[\alpha]^{24}_{D}$ −45.4° (*c* 0.28, MeOH); UV λ_{max} MeOH (ϵ) 219 (18 200) nm; IR (KBr) ν_{max} 3500, 3420, 3060, 2960, 2915, 2870, 1730, 1715, 1605, 1505, 1465, 1370, 1240, 1180, 1070, 855 cm^{-1}; ^1H and ^{13}C NMR (partial), see Tables 1 and 2; TLC 1,2-dichloroethane−MeOH (9:1), system A, R_f 0.57, hexane−$CHCl_3$−EtOAc−MeOH (2:6:1.5:0.5), system B, R_f 0.65; HPLC condition 1, t_R 69.7 min; condition 2, t_R 69.6 min.

(2″*S*,3″*R*)-Dibromo-7-*epi*-cephalomannine (4): mp 163−165 °C, $[\alpha]^{21}_{D}$ −44.1° (*c* 0.29, MeOH); UV λ_{max} MeOH (ϵ) 218 (20 000) nm; IR (KBr) ν_{max} 3500, 3420, 3060, 2960, 2915, 2870, 1730, 1715, 1605, 1505, 1465, 1370, 1240, 1180, 1070, 855 cm^{-1}; ^1H and ^{13}C NMR (partial) see Tables 1 and 2; TLC system A, R_f 0.54, system B, R_f 0.63; HPLC condition 1, t_R 71.9 min, condition 2, t_R 72.6 min.

(2″*R*,3″*S*)-Dibromocephalomannine (5): mp 185−187 °C; $[\alpha]^{24}_{D}$ −41.2° (*c* 0.32, MeOH), UV λ_{max} MeOH (ϵ) 226 (14 700) nm; IR (KBr) ν_{max} 3500, 3420, 3060, 2960, 2915, 2870, 1730, 1715, 1605, 1505, 1465, 1370, 1240, 1180, 1070, 855 cm^{-1}; ^1H and ^{13}C NMR (partial), see Tables 1 and 2; FABMS *m*/*z* $[M + K]^+$ 1030 (3), $[M + Na]^+$ 1014 (4), $[M + H]^+$ 992 (4), $[M - BzOH - H_2O - 2H]^+$ 854 (2), $[T]^+$ 569 (10), $[T-H_2O]^+$ 551 (4), $[T - AcOH]^+$ 509 (17), $[SBr + 2H]^+$ 424 (9), $[SBr - H_2O]^+$ 404 (5), $[T - AcOH - BzOH]^+$ 387 (13), $[SBr - CO_2\text{-}2H]^+$ 376 (63), $[SBr - CO_2 - CHO]^+$ 348 (12); HRFABMS *m*/*z* found 990.191 10, calcd for $C_{45}H_{54}NO_{14}$-$^{79}Br_2$ $[M + H]^+$, 990.191 00,; TLC system A, R_f 0.37, system B, R_f 0.30; HPLC condition 1, t_R 43.8 min, condition 2, t_R 46.6 min.

(2″*S*,3″*R*)-Dibromocephalomannine (6): mp 171−173 °C; $[\alpha]^{24}_{D}$ −44.4° (*c* 0.30, MeOH); UV λ_{max} MeOH (ϵ) 226 (12 400) nm; IR (KBr) ν_{max} 3500, 3420, 3060, 2960, 2915, 2870, 1730, 1715, 1605, 1505, 1465, 1370, 1240, 1180, 1070, 855 cm^{-1}; ^1H and ^{13}C NMR (partial), see Tables 1 and 2; TLC system A, R_f 0.34, system B, R_f 0.28; HPLC condition 1, t_R 45.8 min, condition 2, t_R 48.4 min.

Chlorination Reaction. A solution of 5 g of the extract of *T. yunnanensis* in 200 mL of 1,2-dichloroethane was added dropwise to a stirred 0.06% solution of chlorine in the same solvent (1250 mL) cooled in an ice bath and kept in the dark. After complete addition (2 h) and an additional hour, HPLC analysis (FSP column) indicated that the cephalomannine (**2**) peak was almost completely eliminated. At the end of the reaction the molar ratio chlorine/cephalomannine was 6.3. The reaction mixture was quenched with 1 L of 0.2% Na_2SO_3 solution and washed with water (2 × 1 L). The aqueous phase was extracted twice with 500 mL of 1,2-dichloroethane. The combined organic layers were dried (Na_2SO_4) and evaporated under reduced pressure to obtain 5.3 g of a solid mixture. This was dissolved in acetone (50 mL), and hexane was added slowly and under stirring, until crystallization began to occur. After standing in the dark at 4 °C for 60 h, the crystals were filtered, washed with cold 20% acetone in hexane, and dried under vacuum to yield 3.1 g of paclitaxel (**1**) (95% pure). Further crystallization of this material, by dissolving in acetone and subsequent additions of hexane and standing overnight at 4 °C, yielded 2.5 g of paclitaxel (98.9% pure). The mother liquors from crystallization of **1** were combined and gave, after rotary evaporation, 2.1 g of a mixture of **7**−**10**.

Separation of Dichlorocephalomannines 7−10 by HPLC. The final purification of compounds **7**−**10** was performed by means of preparative HPLC on a Waters Deltapak C_{18} column (19 mm × 30 cm) with MeCN−H_2O (45:55) at 15 mL/min as solvent. Portions (200 mg each) of the mixture **7**−**10** dissolved in methanol (2 mL) were injected. Elution times were approximately 86 (**9**), 98 (**10**), 118 (**7**), and 124 min (**8**). Ten consecutive injections and collection of the eluates yielded, after removal of the solvent under reduced pressure, compounds **7** (8 mg), **8** (5 mg), **9** (32 mg), and **10** (41 mg), as white crystals. For analytical HPLC the same columns and conditions were employed as for compounds **3**−**6**.

(2″*R*,3″*R*)-Dichlorocephalomannine (7): mp 178−181 °C; $[\alpha]^{23}_{D}$ −38.9° (*c* 0.24, MeOH); UV λ_{max} MeOH (ϵ) 218sh (16 000), 228 (17 200) nm; IR (KBr) ν_{max} 3500, 3400, 3110, 3060, 2965, 2915, 2870, 1730, 1715, 1605, 1505, 1465, 1370, 1240, 1180, 1070, 855, 710 cm^{-1}; ^1H and ^{13}C NMR (partial), see Tables 3 and 4; TLC system A, R_f 0.49, system B, R_f 0.44; HPLC: condition 1, t_R 48.3 min, condition 2, t_R 46.0 min.

(2″*S*,3″*S*)-Dichlorocephalomannine (8): mp 160−162 °C; $[\alpha]^{22}_{D}$ −40.2° (*c* 0.29, MeOH); UV λ_{max} MeOH (ϵ) 215 (14 100), 228 (14 700) nm; IR (KBr) ν_{max} 3500, 3400, 3110, 3060, 2965, 2915, 2870, 1730, 1715, 1605, 1505, 1465, 1370, 1240, 1180, 1070, 855, 710 cm^{-1}; ^1H and ^{13}C NMR (partial), see Tables 3 and 4; TLC system A, R_f 0.46, system B, R_f 0.39; HPLC condition 1, t_R 49.7 min, condition 2, t_R 48.0 min.

(2″*R*,3″*S*)-Dichlorocephalomannine (9): mp 190−192 °C; $[\alpha]^{23}_{D}$ −56.9° (*c* 0.45, MeOH); UV λ_{max} MeOH (ϵ) 226 (14 800) nm; IR (KBr) ν_{max} 3500, 3400, 3110, 3060, 2965, 2915, 2870, 1730, 1715, 1605, 1505, 1465, 1370, 1240, 1180, 1070, 855, 710 cm^{-1}; ^1H and ^{13}C NMR (partial), see Tables 3 and 4; FABMS *m*/*z* $[M + K]^+$ 940 (6), $[M + Na]^+$ 924 (3), $[M + H]^+$ 902 (10), $[T]^+$ 569 (6), $[T - AcOH]^+$ 509 (18), $[T - PhCOOH - AcOH]^+$ 387

(11), $[SCl + H]^+$ 333 (20), $[T - PhCOOH - 2\ AcOH]^+$ 327 (21), $[SCl - CO_2]^+$ 288 (100), $[SCl - CO_2 - CH_2O]^+$ 258 (43); TLC system A, R_f 0.43, system B, R_f 0.36; HPLC condition 1, t_R 38.5 min, condition 2, t_R 37.7 min.

(2″*S*,3″*R*)-Dichlorocephalomannine (10): mp 186−188 °C; $[\alpha]^{23}{}_D$ −45.9° (*c* 0.25, MeOH); UV λ_{max} MeOH (ϵ) 227 (15 000) nm; IR (KBr) ν_{max} 3500, 3400, 3110, 3060, 2965, 2915, 2870, 1730, 1715, 1605, 1505, 1465, 1370, 1240, 1180, 1070, 855, 710 cm^{-1}; ^1H and ^{13}C NMR (partial), see Tables 3 and 4; TLC system A, R_f 0.41, system B, R_f 0.33; HPLC condition 1, t_R 41.7 min, condition 2, t_R 41.8 min.

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