An Improved Method for the Separation of Paclitaxel and Cephalomannine

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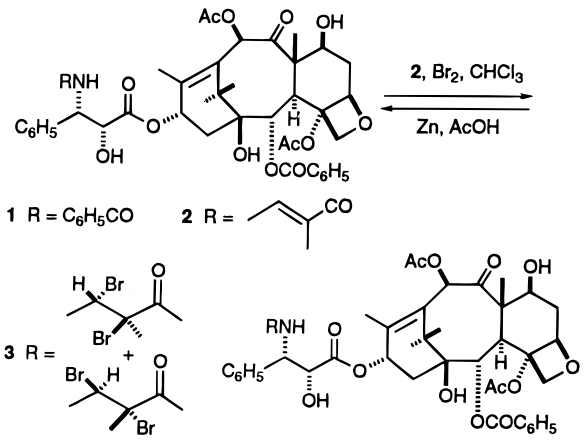
Treatment of a mixture of paclitaxel and cephalomannine with bromine under mild conditions yields a readily separable mixture of paclitaxel and 2″,3″-dibromocephalomannine. Cephalomannine can be regenerated by treating 2″,3″-dibromocephalomannine with zinc in acetic acid.

The complex diterpenoid paclitaxel (Taxol) (**1**) continues to be of great interest, both clinically and chemically, because of its demonstrated clinical activity against both ovarian and breast cancers.[1−4] Paclitaxel occurs in various parts, but especially in the bark and the needles of several yew species, including *Taxus brevifolia*, *Taxus baccata*, and *Taxus wallichiana*. Its isolation from these natural sources is a relatively difficult and expensive process, in part because of the relatively small amounts of paclitaxel present (typically about 0.01%) but also because of the presence of a closely related congener, cephalomannine (**2**).[5,6] Paclitaxel and cephalomannine occur in differing amounts and ratios, depending on the plant part and species, but various surveys have placed the paclitaxel content in the range of 0.001−0.08% and the cephalomannine content at 0.001−0.22%.[7−9] It should be noted, however, that the highest concentration of paclitaxel was observed in *T. brevifolia* bark, [8] which can be harvested only with concomitant destruction of the tree, while the highest concentration of cephalomannine was observed in a fall shoot collection of *Taxus media*; [7] furthermore, the mean concentration of cephalomannine observed in the renewable fall shoot collections was higher than the mean concentration of paclitaxel for four different *Taxus* species.[7]

Because cephalomannine is so closely related to paclitaxel, it also has very similar chromatographic properties and is thus rather difficult to separate from paclitaxel. Several chromatographic methods for the separation of paclitaxel and cephalomannine have been published,[10−13] but these primarily rely on reversed-phase chromatography or on the use of expensive bonded-phase columns and are thus not readily adaptable to large-scale operations. Although paclitaxel can now be prepared from baccatin III,[14] and this is the route used for the commercial production of paclitaxel by one major pharmaceutical company,[14,15] the direct isolation of paclitaxel is still a matter of practical interest in other situations. An improved method for the separation of paclitaxel and cephalomannine thus continues to be a matter of some importance.

A few years ago, we published a simple method for the separation of paclitaxel and cephalomannine.[16] This method works very well, but it uses the toxic and rather expensive reagent osmium tetraoxide, and it yields a product (cephalomannine diol) that cannot readily be converted back to cephalomannine. Thus, while the method allows the ready preparation of pure paclitaxel, it does not allow the similarly ready preparation of pure cephalomannine. We now report an alternate method for the simple separation of paclitaxel and cephalomannine that avoids the use of toxic and expensive reagents and allows the regeneration of cephalomannine.

Treatment of a mixture of paclitaxel and cephalomannine with bromine in $CHCl_3$ at room temperature gave a clean conversion to a mixture of unchanged paclitaxel and 2″,3″-dibromocephalomannine (**3**) if the reaction was monitored and stopped within 5 min. Allowing the reaction mixture to stand in the presence of excess bromine for periods longer than 5 min led to the formation of additional products that were not investigated but which presumably derived from reactions such as opening of the oxetane ring. The resulting mixture of paclitaxel and 2″,3″-dibromocephalomannine could readily be separated by chromatography on an economical Si gel column to give the two pure products in about 95% overall yield. In alternate procedures, cephalomannine (or a mixture of paclitaxel and cephalomannine) could be converted to 2″,3″-dibromocephalomannine with either pyridinium perbromide or tetrabutylammonium tribromide.



2″,3″-Dibromocephalomannine (**3**) was obtained as a mixture of diastereomers, as indicated by the doubling of certain peaks in its ^{1}H-NMR spectrum. Treatment of this mixture, however, with zinc and acetic acid at room temperature resulted in debromination to regenerate cephalomannine, which was obtained as a homogeneous product in 92% yield, identical to the pure natural product. The formation of a single product from the diastereomeric dibromides is, of course, explicable on the basis of a stereoselective anti-addition of bromine followed by a stereoselective anti-elimination of bromine by zinc.[17]

This work provides a simple and economical method for the separation of paclitaxel and cephalomannine and for the production of pure cephalomannine.

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Experimental Section

General Experimental Procedures. The general experimental procedures have been previously described by Chaudhary *et al.*[18]

Preparation of 2″,3″-Dibromocephalomannine (3) and Paclitaxel from a Mixture of Cephalomannine and Paclitaxel. A mixture of cephalomannine and paclitaxel (300 mg, 30:70 ratio as determined by ^{1}H-NMR spectroscopy) was dissolved in $CHCl_3$ (10 mL), and Br_2 (0.05 mL, 0.96 mmol) was added dropwise over 1 min at room temperature. After being stirred for 5 min, the mixture was diluted with CH_2Cl_2 (20 mL), washed with 10% aqueous $Na_2S_2O_3$ and then with H_2O and brine, and then dried (Na_2SO_4) and evaporated. The crude product (327 mg) was separated by chromatography on Si gel with elution with hexane:EtOAc, 6:4, to give 2″,3″-dibromocephalomannine (**3**) (101 mg, 95%) in the early fractions and paclitaxel (**1**) (201 mg, 95%) in the later fractions. 2″,3″-Dibromocephalomannine: ^{1}H-NMR ($CDCl_3$) δ 1.15 (s, 3H, H_3-16), 1.23 and 1.25 (two s, 3H total, H_3-17), 1.67 (s, 3H, H_3-19), 1.74 (m, 3H, H_3-4″), 1.80 (s, 3H, H_3-18) 1.87 (m, 1H, H-6β), 1.98 and 1.99 (two s, 3H total, H_3-5″), 2.25 (s, 3H, 10-$OCOCH_3$), 2.34 and 2.35 (two s, 3H total, 4-OAc), 2.54 (m, 1H, H-6α) 3.77 (d, J = 7.0 Hz, 1H, H-3), 4.18 (d, J = 8.4 Hz, 1H, H-20β), 4.29 (d, J = 8.4 Hz, 1H, H-20α), 4.39 (m, 1H, H-7), 4.61 (q, J = 6.6 Hz, 1H, H-3″), 4.72 (m, 1H, H-2′), 5.53, 5.57 (overlapping dd, J = 8.9, 2.3 Hz, 1H, H-3′) 5.66 (d, J = 7.0 Hz, 1H, H-2), 6.20 (t, J = 8.8 Hz, 1H, H-13), 6.28 (s, 1H, H-10), 7.30−8.12 (m, 11H, ArH and NH); HRFABMS calcd for $C_{45}H_{54}{}^{79}Br^{81}BrNO_{14}$ m/z 992. 1991, found 992.1929.

Reaction of Cephalomannine with Pyridinium Perbromide. Cephalomannine (90% pure, 30 mg) was dissolved in $CHCl_3$ (1 mL) and treated with pyridinium perbromide (58 mg). After 30 min, the solution was loaded directly onto a prep.TLC plate and developed with hexane:EtOAc, 2:3. 2″,3″-Dibromocephalomannine was eluted as a chromatographically homogeneous compound (26.5 mg, 93%), identical to the sample previously described.

Reaction of Cephalomannine with Tetrabutylammonium Tribromide. Cephalomannine (90% pure, 30 mg) in $CHCl_3$ (1 mL) was treated with n-Bu_4NBr_3 (88 mg) for 1 h at room temperature. Purification as described above gave 2″,3″-dibromocephalomannine (24 mg, 83%).

Preparation of Cephalomannine from 2″,3″-Dibromocephalomannine. 2″,3″-Dibromocephalomannine (30 mg) in AcOH (0.5 mL) was treated with freshly activated zinc (38 mg) with stirring for 2 h. The solution was then diluted with EtOAc (10 mL), washed with saturated aqueous $NaHCO_3$ (2 × 10 mL), H_2O (1 × 10 mL), and brine (1 × 10 mL), dried ($MgSO_4$), and evaporated. The crude product was purified by prep.TLC (EtOAc:hexane, 3:2) to give cephalomannine (22 mg, 88%). ^{1}H NMR δ 1.26 (s, 3H, H_3-17), 1.68 (s, 3H, H_3-19), 1.72 (d, J = 7.0, H_3-4″), 1.79 (s, 3H, H_3-18), 1.80 (s, 3H, H_3-5″), 2.24 (s, 3H, 10-$OCOCH_3$), 2.36 (s, 3H, 4-$OCOCH_3$), 2.54 (m, 1H, H-6), 3.78 (d, J = 7.0 Hz, 1H, H-3), 4.29 and 4.18 (ABq, J = 8.4, 2H, H_2-20), 4.40 (m, 1H, H-7), 4.41 (dd, J = 5.3, 2.7 Hz, 1H, H-2′), 4.93 (br d, J = 7.78 Hz, 1H, H-5), 5.61 (dd, J = 8.8, 2.7 Hz, 1H, H-3′), 5.67 (d, J = 7.0 Hz, 1H, H-2), 6.21 (br t, J = 8.4 Hz, 1H, H-13), 6.27 (s, 1H, H-10), 1.15 (s, 3H, H_3-16), 6.44 (qd, J = 7.0, 1.2, H-3″), 6.51 (d, J = 8.9 Hz, 1H, NH), 7.30−8.12 (m, 10H, 2-OCOPh, 3′-Ph).

References and Notes

(1) For recent reviews of the clinical use of paclitaxel, see: McGuire, W. P.; Rowinsky, E. K., Eds. *Paclitaxel in Cancer Treatment*; M. Dekker: New York, 1995; pp 1−337.
(2) Georg, G. I., Chen, T. T., Ojima, I., Vyas, D. M., Eds. *Taxane Anticancer Agents: Basic Science and Current Status*; ACS Symposium Series No. 583; American Chemical Society: Washington, DC, 1994, pp 1−339.
(3) Kingston, D. G. I. *Trends Biotechnol.* **1994**, *12*, 222−227.
(4) Suffness, M., Ed. *Taxol: Science and Applications*; CRC Press: Boca Raton, FL, 1995; pp 1−415.
(5) Powell, R. G.; Miller, R. W.; Smith, C. R., Jr. *J. Chem. Soc., Chem. Commun.* **1979**, 102−104.
(6) Miller, R. W.; Powell, R. G.; Smith, C. R., Jr.; Arnold, A.; Clardy, J. *J. Org. Chem.* **1981,** *46*, 1469−1474.
(7) Wheeler, N. C.; Jech, K.; Masters, S.; Brobst, S. W.; Albarado, A. B.; Hoover, A. J.; Snader, K. M. *J. Nat. Prod.* **1992**, *55*, 432−440.
(8) Kelsey, R. G.; Vance, N. C. *J. Nat. Prod.* **1992**, *55*, 912−917.
(9) Witherup, K. M.; Look, S. A.; Stasko, M. W.; Ghiorzi, T. J.; Muschik, G. M. *J. Nat. Prod.* **1990**, *53*, 1249−1255.
(10) Cardellina, J. H., II *J. Liq. Chromatogr.* **1991,** *14*, 659−665.
(11) Richheimer, S. L.; Tinnermeier, D. M.; Timmons, D. W. *Anal. Chem.* **1992**, *64*, 2323−2326.
(12) Wickremesinhe, E. R. M.; Arteca, R. N. *J. Liq. Chromatogr.* **1993**, *16*, 3263−3274.
(13) Witherup, K. M.; Look, S. A.; Stasko, M. W.; McCloud, T. G.; Issaq, H. J.; Muschik, G. M. *J. Liq. Chromatogr.* **1989**, *12*, 2117−2132.
(14) Holton, R. A.; Biediger, R. J.; Boatman, P. D. In *Taxol: Science and Applications*; Suffness, M., Ed.; CRC Press: Boca Raton, FL, 1995; pp 97−121.
(15) McNeil, C. *J. Natl. Cancer Inst.* **1995**, *87*, 1106−1108.
(16) Kingston, D. G. I.; Gunatilaka, A. A. L.; Ivey, C. A. *J. Nat. Prod.* **1992**, *55*, 259−261.
(17) House, H. O.; Ro, R. S. *J. Am. Chem. Soc.* **1958**, *80*, 182−187.
(18) Chaudhary, A. G.; Chordia, M. D.; Kingston, D. G. I. *J. Org. Chem.* **1995**, *60*, 3260−3262.

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