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MODIFIED TAXOLS, 7[1]. A METHOD FOR THE SEPARATION OF TAXOL AND CEPHALOMANNINE

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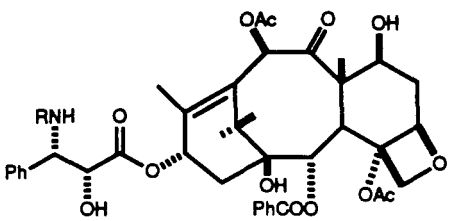
ABSTRACT.—Taxol [**1**] can be separated from the closely related co-occurring diterpenoid cephalomannine [**2**] by oxidation of a mixture of the two with OsO_4 and flash chromatography of the resulting products.

The diterpenoid natural product taxol [**1**], first isolated by Wall and his collaborators (1), is of great current interest because of its clinically demonstrated efficiency against ovarian cancer (2,3). The major problem preventing large-scale use of taxol is that of supply, as summarized in a recent review on the chemistry of taxol (4). Taxol is currently isolated from the bark of *Taxus brevifolia*, but the yield has been as low as 0.007%, and the supply of bark is restricted by the fact that *T. brevifolia* is indigenous to the ecologically threatened old-growth forests of the Pacific northwest (4). Although other approaches to taxol production, such as hemisynthesis from baccatin III (5,6) and plant tissue culture (7) are promising for the future, the production of taxol for the next few years will likely remain tied to extraction from *T. brevifolia* bark or from an alternate source such as *Taxus* needles (4).



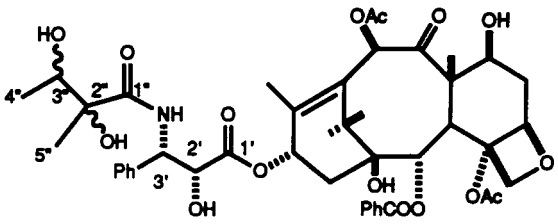
1 R=C_6H_5CO
2 R=MeCH=CCO
|
Me

The isolation of taxol from *T. brevifolia* bark or comparable biological sources is complicated not only by the small amount of taxol present in these sources but also by the fact that taxol frequently co-occurs with the closely related diterpenoid cephalomannine [**2**] (8).

The separation of taxol from cephalomannine is a difficult process, requiring either very careful chromatography under low resolution conditions [separation of taxol from cephalomannine on a large scale has been carried out by Polysciences, Inc., using a CH_2Cl_2/*n*-BuOH solvent system on Si gel (Dr. F. Boettner, personal communication)] or separation by hplc (9,10), and this separation adds significantly to the cost of preparing taxol on a large scale for use as an anticancer drug. Thus an alternative separation method that does not require such chromatography would be desirable.

Cephalomannine [**2**] differs from taxol [**1**] only in the nature of the 3′-*N*-acyl group; in taxol this is a benzoyl group, while in cephalomannine it is a tigloyl group. The presence of a simple double bond in the cephalomannine side-chain does, however, provide an opportunity for selective chemical modification. Reaction of a mixture of cephalomannine and taxol with OsO_4 under mild conditions resulted in rapid and selective formation of a diastereomeric mixture **3** of diol derivatives of cephalomannine; taxol was unchanged under these conditions, since the Δ^{11} double bond of the taxene ring is sterically hindered and thus inaccessible

[1]For Part 6, see Z. Zhao, D.G.I. Kingston, and A.R. Crosswell, *J. Nat. Prod.*, **54**, 1607 (1991).



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to most reagents. The resulting mixture of taxol and cephalomannine diols could be readily separated by simple flash chromatography on Si gel, giving taxol in good yield.

The reaction can also be run by a catalytic method, to reduce the cost and toxicity problems associated with OsO_4. In this method, a catalytic amount of OsO_4 in the presence of *t*-butylhydroperoxide and tetraethyl ammonium acetate (11) is used. Reaction again proceeds cleanly to give the diols **3** and unreacted taxol [**1**], which can be separated by flash chromatography to give taxol in excellent yield.

This method thus provides a simple and efficient procedure for the isolation of taxol in pure form from mixtures of taxol and cephalomannine.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—The general procedures used were as previously described (12). The ^{1}H- and ^{13}C-nmr spectra were recorded on a Varian Unity 400 spectrometer at 400 and 100.57 MHz, respectively. The ^{1}H- and ^{13}C-nmr signals for the baccatin III ring system of **3** were essentially identical to those reported for cephalomannine (8,13) except that some signals appeared as doublets since **3** is a mixture of diastereoisomers.

HYDROXYLATION OF CEPHALOMANNINE IN A MIXTURE OF TAXOL AND CEPHALOMANNINE WITH A STOICHIOMETRIC AMOUNT OF OSO_4.—To a stirred solution of a mixture of cephalomannine and taxol (66:34 by ^{1}H nmr, 20 mg) in pyridine (1 ml) was added a solution of OsO_4 in pyridine (19.6 mM, 1.5 ml), and the mixture was stirred at 25° for 12 h, after which a solution of $NaHSO_3$ (10% in H_2O, 5 ml) was added and stirred for a further 2 h. The solution was acidified to pH 1 with 3 N HCl and extracted with EtOAc (3 × 5 ml). The organic extracts were washed with brine (2 × 5 ml), dried (Na_2SO_4), and evaporated to yield a crude mixture (19 mg) of taxol [**1**] and cephalomannine diols **3**.

CATALYTIC METHOD OF HYDROXYLATION.—A crude mixture of cephalomannine [**2**] and taxol [**1**] was determined to consist of ca. 80% (**1**+**2**) by weight by preparative tlc of a known amount and recovery of the relevant band. To a stirred solution of this crude mixture (1.0 g) and $Et_4NOAc{\cdot}4H_2O$ (80 mg) in Me_2CO (hplc grade, 10 ml) at 0° was added a solution of *t*-BuOOH (70% in H_2O; 300 μl) followed by a solution of OsO_4 (2.5% in *t*-BuOH, 300 μl). After stirring for 1 h at 0°, the cooling bath was removed and the mixture stirred for 1 h at 25° until reaction was complete, as judged by tlc. The reaction mixture was cooled to 0°, and 30 ml of CH_2Cl_2 was added followed by a freshly prepared solution of aqueous $NaHSO_3$ (10%, 10 ml). The cooling bath was removed, and the mixture was stirred for 1 h and then transferred to a separatory funnel. The aqueous phase was extracted (5×) with aliquots (20 ml) of CH_2Cl_2. The combined organic extracts were washed with brine, dried (Na_2SO_4), and evaporated to yield a pale yellow solid (901 mg).

SEPARATION OF TAXOL [**1**] FROM CEPHALOMANNINE DIOLS **3** BY FLASH CHROMATOGRAPHY.—The crude reaction product containing **1** and **3** (900 mg) from the catalytic method of hydroxylation was subjected to flash chromatography over a column of Si gel (Merck; 230–400 mesh) using hexanes-EtOAc (50:50) and eluted with increasing amounts of EtOAc in hexanes. The fraction eluted with 60% EtOAc in hexanes afforded taxol [**1**] (570 mg, 89% recovery based on 80% pure starting material); the isolated material was identical (ms, ^{1}H nmr, tlc) with an authentic sample. Those fractions eluted with 80–90% EtOAc in hexanes gave a diastereomeric mixture **3** of cephalomannine diols (150 mg, 94% recovery): ir (KBr) 3420, 2980, 1723, 1656, 1586, 1522, 1453, 1384, 1316, 1245, 1180, 1096, 1071, 1025, 981, 949, 904, 776, 711 cm^{-1}; fabms *m/z* $[MNa]^+$ 888, $[MH]^+$ 866; ^{1}H nmr (side-chain protons only) 1.26 (3H, d, J=7, Me-5″), 1.30 (3H, s, Me-4″), 4.00 (1H, m, H-3″), 4.64 and 4.69 (1H, each d, J=3, H-2′), 5.50 (1H, m, H-3′); ^{13}C nmr (side-chain carbons only) 175.98 (C-1″), 173.87 and 172.73

(C-1′), 73.31 and 73.20 (C-2′), 72.21 and 72.18 (C-2″), 71.59 and 71.01 (C-3″), 54.84 and 54.75 (C-3′), 22.49 and 22.10 (C-4″), 16.82 and 16.01 (C-5″).

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