Cephalomannine; a New Antitumour Alkaloid from *Cephalotaxus mannii*

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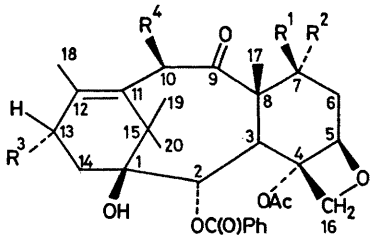
Summary Chemical investigation of the antileukaemic and cytotoxic principles of *Cephalotaxus mannii* has led to the isolation and characterization of cephalomannine, an alkaloidal taxane derivative structurally unrelated to the harringtonine series of alkaloids characteristic of most *Cephalotaxus* species.

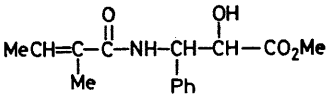
IN a search for antitumour agents from plant sources, we have isolated a new alkaloid, cephalomannine (**1**), from the stems and roots of *Cephalotaxus mannii*,† a tree found in the Shillong Forest of India. The alkaloid (**1**) is cytotoxic in KB cell culture, $LD_{50} = 3{\cdot}8 \times 10^{-3}$ μg ml^{-1}, and shows potent inhibition of PS leukaemia in mice.[1] Taxol (**2**) is also present along with baccatin III (**3**). Taxane diterpenes including (**2**) and (**3**) have previously been reported only in the genus *Taxus*,[2,3] while other known antitumour alkaloids of *Cephalotaxus* have all been derivatives of cephalotaxine.[4]



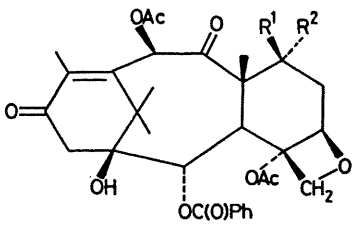
(**1**); R^1 = OH, R^2 = H, R^3 = OC(O)CH(OH)·CHPh.NH.CO.-C(=CHMe)Me, R^4 = OAc
(**2**); R^1 = OH, R^2 = H, R^3 = OC(O)CH(OH).CHPh.-NH.C(O)Ph, R^4 = OAc
(**3**); $R^1 = R^3$ = OH, R^2 = H, R^4 = OAc
(**4**); $R^1 = R^3 = R^4$ = OH, R^2 = H
(**5**); R^1 = H, $R^2 = R^3$ = OH, R^4 = OAc
(**6**); R^1 = H, $R^2 = R^3 = R^4$ = OH

An alcoholic extract of the ground plant material was concentrated, defatted by partition between light petroleum and water, and the aqueous phase was then extracted with chloroform. Cephalomannine (**1**) was isolated from the chloroform-soluble material by a succession of operations that included chromatography on silica, two successive countercurrent distributions (solvent system: ethyl acetate–hexane–methanol–water), and finally h.p.l.c. on a C_{18} μ Bondapak column; this procedure also provided compounds (**2**) and (**3**). Crystallization of (**1**) from aqueous methanol gave needles, m.p. 184—186 °C; $[\alpha]_D$ −41° (MeOH); field desorption mass spectrometry gave an apparent M^+ at *m/e* 831 (7%) consistent with the formulation $C_{45}H_{53}NO_{14}$. Compound (**2**), m.p. 198—203 °C, $[\alpha]_D$ −42° (MeOH), was identical in all respects with known taxol,‡ and (**3**), m.p. 229—231 °C, was identical in all respects with baccatin III as reported by earlier workers.[3] ^{1}H N.m.r. signals for (**1**) and (**2**) are similar[2] except that the spectrum of (**1**) indicates five fewer aromatic protons (δ 7·0—7·5); in addition, (**1**) exhibits signals at δ 1·65, 1·85 (2× vinyl Me), and 6·37 (vinyl H) not observed in the spectrum of (**2**). The ^{1}H n.m.r. spectrum of (**3**) is similar to those of (**1**) and (**2**) except for an upfield shift of the C-13 proton signal (δ 6·17 to 4·85) and other differences due to absence of the nitrogen containing ester grouping.

Both (**1**) and (**3**) are relatively labile when dissolved in 1% sodium bicarbonate in methanol–water (3 : 1). Treatment of (**3**) with this reagent at 26 °C for 5 h gave tetrol (**4**) in good yield along with lesser amounts of baccatin V (**5**) and the corresponding tetrol (**6**). Under identical conditions, (**1**) gave a complex mixture including (**3**)—(**7**), all



(**7**)



(**8**); R^1 = OH, R^2 = H

separable by t.l.c. Compound (**3**) was also oxidized with activated manganese dioxide to give the ketone (**8**), m.p. 210—212 °C, which exhibited the expected singlet at δ 6·45 due to the C-10 proton.[2,3] From previous reports compound (**2**) is a derivative of (**3**). Since (**1**) and (**3**) each give (**4**), (**5**), and (**6**), compounds (**1**) and (**2**) likewise must contain the same diterpene ring system.

The methyl ester (**7**) was also obtained by treating (**1**) with sodium methoxide in methanol under anhydrous conditions; we were unable to isolate the other expected product (**4**) from this reaction mixture.[2] The ester (**7**) was characterized as follows: m.p. 129—132 °C; ν_{max} ($CHCl_3$) 3480, 3410 (OH, NH), 1710 (ester CO), 1640 (amide CO), and 1605 (C=C) cm^{-1}; δ 1·72 (3H, d, *J* 7 Hz, vinyl Me), 1·82 (3H, s, vinyl Me), 3·78 (3H, s, ester Me), 4·52 (1H, d, *J* 2 Hz, 2′H), 5·54 (1H, ABq, *J* 2, 8 Hz, 3′-H), 6·48 (1H, q, *J* 7 Hz, vinyl H), 6·5 (1H, br d, *J* 8 Hz, NH), and 7·0—7·5 (5H, m, ArH); *m/e* ($M^+ - CO_2Me$) calc. 218·1155, found 218·1168.

Characterisation of the ester (**7**) and the alcohols (**3**)—(**6**), has enabled us to establish the indicated structure (**1**) for cephalomannine. One remaining doubtful point is the stereochemistry of the α-methylcrotonate unit in (**1**) and (**7**).

Formation of the methyl ester (**7**) under mild solvolytic conditions may be surprising since α-substituted allylic esters usually undergo alkyl–oxygen cleavage instead of cleavage by the more common acyl–oxygen mechanism. The anomalous course of this reaction may be explained by two factors. Steric constraints could preclude development of a planar carbonium ion at C-11 so that alkyl–oxygen cleavage at C-13 is not facilitated; furthermore,

† Plant material supplied by Dr. R. E. Perdue, Jr., U.S. Department of Agriculture. *Added in proof*: In view of our chemical results, the botanical classification of our plant material is being re-evaluated.

‡ A reference sample of taxol was generously supplied by Dr. J. Douros, Developmental Therapeutics Program, National Cancer Institute, Bethesda, Maryland 20014.

an α-hydroxy substituent is available in the leaving group to facilitate interesterification by a $B_{Ac}2$ mechanism. Ready epimerization of the secondary hydroxy group at C-7 was unexpected and may proceed by transannular interaction with the C-4 acetate group through an ortho-ester intermediate. Careful manipulation is necessary with this series of compounds in order to avoid unwanted artifacts.

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