19-HYDROXYBACCATIN III, 10-DEACETYLCEPHALOMANNINE, AND 10-DEACETYLTAXOL: NEW ANTITUMOR TAXANES FROM *TAXUS WALLICHIANA*

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ABSTRACT.—Activity-guided, chromatographic fractionation of a polar extract of *Taxus wallichiana* Zucc. (originally identified as *Cephalotaxus mannii* Hook.) resulted in the isolation of three new KB cytotoxic taxane derivatives. Nmr and ms spectral analyses permitted their characterization as 19-hydroxybaccatin III (**3**), 10-deacetylcephalomannine (**4**), and 10-deacetyltaxol (**5**). The latter two compounds, which are also active against PS leukemia *in vivo*, were observed to be especially labile, each forming equilibrium mixtures with their cytotoxic C-7 epimers (**9**, **10**).

The genus *Cephalotaxus* (Cephalotaxaceae) incorporates a number of yew-like evergreen trees and shrubs native to southeastern Asia. *C. harringtonia* (Forbes) K. Koch var. *harringtonia* (1) and other varieties and species of *Cephalotaxus* (2) contain four cephalotaxine esters (harringtonine, isoharringtonine, homoharringtonine, and deoxyharringtonine) which possess exceptional antitumor activities (3, 4), and the synthesis and biosynthesis of these unusual alkaloids have attracted much recent attention (5, 6).

In a preliminary communication from this laboratory (7), plant material from the Shillong forest of India, originally identified as *C. mannii* Hook., failed to yield the cephalotaxine alkaloids but, instead, yielded antitumor taxanes characteristic of the genus *Taxus* (Taxaceae) (8). Confusing morphological similarities between *C. mannii* and *T. wallichiana* Zucc. had caused preliminary misidentification, and our plant material is now considered to be the latter species.[4] Thus, the presence of the active taxane esters, taxol (**1**) and cephalomannine (**2**), led to reconsideration of the botanical identity of the plant material (9). Phytochemical work on the *Taxus* alkaloids and other taxane derivatives has recently been reviewed (10).

Our previous work with *T. wallichiana* began with a large-scale ethanolic extraction of the roots, stems, and needles (9). The concentrated ethanolic extract was diluted with water and defatted by partitioning with petroleum ether; the residue, in turn, was extracted with chloroform. The KB active chloroform extract was then chromatographed on two successive silica gel columns developed with chloroform-methanol, and fractions were combined on the basis of similar tlc patterns. The KB bioassay indicated a number of active fractions. A fraction (F216) more polar than taxol and cephalomannine, upon tlc and hplc analysis, was shown to be free of the known active taxanes; and, because it was significantly active (KB <1 μg/ml, PS 136% T/C at 150 mg/kg), this fraction was the starting point for the current investigation.

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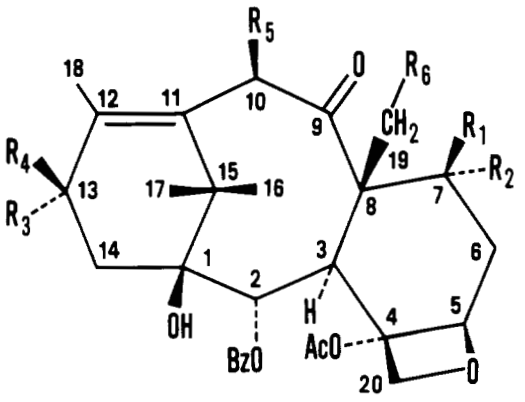
[3]The mention of firm names or trade products does not imply that they are endorsed or recommended by the U.S. Department of Agriculture over other firms or similar products not mentioned.

[4]Personal communication, Dr. Richard H. Eyde, Curator, Department of Botany, Smithsonian Institution, Washington, D.C., November 27, 1978.



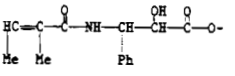
1 taxol: R_1 = OH, R_2 = R_4 = R_6 = H, R_3 = BzNH-CH(Ph)-CH(OH)-C(=O)-O-(3'-2'-1'), R_5 = OAc.

2 cephalomannine: R_1 = OH, R_2 = R_4 = R_6 = H,

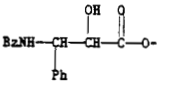
R_3 = HC(Me)=C(Me)-C(=O)-NH-CH(Ph)-CH(OH)-C(=O)-O-(3"-2"-1"-3'-2'-1'), R_5 = OAc

3 19-hydroxybaccatin III: R_1 = R_3 = R_6 = OH, R_2 = R_4 = H, R_5 = OAc.

4 10-deacetylcephalomannine: R_1 = R_5 = OH, R_2 = R_4 = R_6 = H, R_3 =



5 10-deacetyltaxol: R_1 = R_5 = OH, R_2 = R_4 = R_6 = H, R_3 =

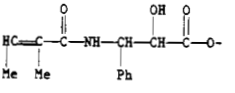


6 baccatin III: R_1 = R_3 = OH, R_2 = R_4 = R_6 = H, R_5 = OAc

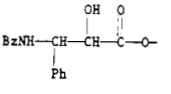
7 13-oxo derivative of baccatin III: R_1 = OH, R_2 = R_6 = H, R_3 + R_4 = O, R_5 = OAc.

8 baccatin V: R_1 = R_4 = R_6 = H, R_2 = R_3 = OH, R_5 = OAc.

9 7-epi-10-deacetylcephalomannine: R_1 = R_4 = R_6 = H, R_2 = R_5 = OH, R_3 =



10 7-epi-10-deacetyltaxol: R_1 = R_4 = R_6 = H, R_2 = R_5 = OH, R_3 =



11 10-deacetylbaccatin III: R_1 = R_3 = R_5 = OH, R_2 = R_4 = R_6 = H.

12 10-deacetylbaccatin V: R_1 = R_4 = R_6 = H, R_2 = R_3 = R_5 = OH.

By column chromatography of F216 on silica gel with a gradient of benzene-ethyl acetate and combining eluted fractions on the basis of tlc analysis, we obtained two crystalline products and two subsequent fractions which possessed the most significant activity (KB, <1 μg/ml). The first crystalline product was identified as 19-hydroxybaccatin III (**3**), a new taxane with moderate KB activity (2.2 μg/ml) comparable with the KB activity of baccatin III (9). The second crystalline product was a new lignan (to be described in a later paper) which was not significantly active (KB, 21 μg/ml). Two other active products were subsequently identified as 10-deacetylcephalomannine, (**4**), and 10-deacetyltaxol, (**5**).

The structural determination of 19-hydroxybaccatin III, (**3**), was greatly simplified by direct comparison of ms and nmr spectra with those of baccatin III, (**6**). The cims (NH_3) revealed an $M+NH_4^+$ peak at m/z 620, a difference of one oxygen from the m/z 604 ($M+NH_4^+$) of baccatin III; in addition, 19-hydroxy-

TABLE 1. ^{1}H nmr chemical shifts for taxanes from *T. wallichiana*.[a]

Protons on	3	4	5	9	10
C-2.....	6.34 d J=7	5.67 d J=7	5.67 d J=7.5	5.73 d J=7.5	5.73 d J=7.5
C-3.....	3.88 d J=7	3.88 d J=7	3.88 d J=7.5	3.91 d J=7.5	3.91 d J=7.5
C-5.....	5.0 m	4.92 br d J=8	4.92 br d J=9	4.90 t J=6	4.90 t J=6
C-6.....	2.6 m	2.2 m	1.9 m	2.3 m	2.3 m
C-7.....	4.4 m	4.3 m	4.18 m	3.66 br t J=3, 12	3.66 br t J=3, 12
C-10.....	6.41 s	5.20 s	5.18 s	5.43 s	5.42 s
C-13	5.0 m	6.17 br t J=9	6.18 br t J=8	6.23 br t J=9	6.23 br t J=9
C-14.....	2.6 m	2.4 m	2.4 m	2.3 m	2.3 m
C-16.....	1.25 s	1.25 s	1.19 s	1.22 s	1.20 s
C-17.....	1.11 s	1.11 s	1.10 s	1.09 s	1.09 s
C-18.....	2.07 d J=1.5	1.77 br s	1.74 br s	1.75 br s	1.74 br s
C-19.....	4.72 ABq J=12	1.73 s	1.74 br s	1.71 s	1.72 br s
C-20.....	4.30 ABq J=8	4.25 ABq	4.25 ABq	4.39 br s	4.39 br s
C-2 OBz....	7.54 m 8.13 dd J=2, 8	7.55 m 8.12 dd J=2, 8	7.47 m 8.12 dd J=2, 8	7.53 m 8.16 dd J=2, 8	7.53 m 8.18 dd J=2, 8
OAc.....	2.26 s 2.29 s	2.34 s	2.37 s	2.47 s	2.51 s
C-2'.....	—	4.72 d J=3	4.78 d J=3	4.72 d J=3	4.79 d J=3
C-3'	—	5.58 dd J=3, 8	5.77 dd J=3, 9	5.62 dd J=3, 9	5.80 dd J=3, 9
C-3' Ph.....	—	7.37 br s	7.43 br s	7.38 br s	7.40 m
C-3' NH....	—	6.77 d J=8	7.14 d J=9	6.59 d J=9	7.02 d J=9
C-3' NBz...	—	—	7.47 m 7.76 dd J=2, 7	–	7.43 m 7.72 dd J=2, 6
C-2" Me.....	—	1.73 s	—	1.75 br s	—
C-3".....	—	6.42 dd J=1, 7	—	6.43 dd J=1.5, 7	—
C-4".....	—	1.7 d	—	1.7 d	—

[a]Chemical shifts (δ) are expressed in ppm from TMS and coupling constants (J) in Hz.

baccatin III showed a strong peak at m/z 602 due to a loss of water ($M+NH_4^+-H_2O$). The eims failed to detect the molecular ion, but the exact mass of the largest fragment agreed well with the expected M^+-OAc peak; the eims baes peak at m/z 105 is characteristic for loss of the benzoate ester from these compounds, and a strong eims peak at m/z 526 ($M^+-OAc-OH$) supported the proposed hydroxybaccatin structure.

In the ^1H nmr spectrum of **3**, the C-19 methyl singlet at 1.67 ppm, characteristic in baccatin III, was replaced by an AB quartet at 4.72 ppm with geminal coupling ($J=12$); this was the only major difference in the proton spectra of the two compounds (table 1). Similarly, the ^{13}C nmr spectra were quite comparable, the most significant difference being a strong downfield shift of the C-19 signal from 9.49 ppm in baccatin III (**6**) to 60.25 ppm for **3**. In table 2 are listed the ^{13}C nmr assignments of 19-hydroxybaccatin III along with those of baccatin III and its C-13 oxidized derivative **7** for comparison; multiplicities observed in a partially

TABLE 2. ^{13}C Nmr chemical shifts (δ) of baccatin III and analogues.

Carbon no.	Baccatin III (**6**)	19-Hydroxy-baccatin III (**3**)	C-13 Oxidized baccatin III (**7**)
1	79.16 (s)	79.35	78.90
2	76.30 (d)	76.43[a]	76.62
3	46.27 (d)	46.92	45.43
4	80.98 (s)	80.85	80.65
5	68.04 (d)	68.04	72.14
6	35.74 (t)	36.39	35.87
7	76.49 (d)	76.43[a]	76.27
8	58.88 (s)	61.55	59.53
9	204.13 (s)	205.63	202.05
10	84.55 (d)	84.62	84.23
11	146.36 (s)	146.42	152.14
12	132.06 (s)	132.06	132.68
13	75.13 (d)	76.43[a]	197.96
14	38.80 (t)	38.28	42.57
15	42.83 (s)	43.09	43.54
16	15.47 (q?)[b]	15.47[b]	13.97[b]
17	22.55 (q?)[b]	22.55[b]	21.77[b]
18	27.04 (q)	26.97	33.27
19	9.49 (q)	60.25	9.16
20	72.33 (t)	72.66	73.11
CO on Ac	171.25, 170.66 (bs)	170.79	170.21
CH_3 on Ac	20.93 (q)	20.73	18.78
CO on Bz	167.15 (s)	167.22	166.96
p-Benzoyl	133.68 (d)	133.62	134.07
o-Benzoyl	130.18 (d)	130.24	130.11
1-Benzoyl	129.59 (s)	129.79	129.58
m-Benzoyl	128.68 (d)	128.62	128.88

[a]Overlapping peaks.
[b]Interchangeable assignments.

decoupled spectrum of baccatin III were helpful in making the assignments. Compared to values for **3** and **6**, the signal for C-11 in **7** is shifted downfield considerably due to the influence of conjugation in the enone grouping.

Each of the two remaining active fractions was rechromatographed on a column of silica gel developed with a gradient of chloroform-methanol; the activity

of the resulting fractions was associated with a single tlc spot. After preliminary purification by preparative tlc, this material gave ^{1}H nmr signals in the aromatic region indicative of a mixture of two new taxanes, one possessing the ester side chain of taxol (**1**) and the other the corresponding side chain of cephalomannine (**2**). Analytical hplc substantiated this conclusion by resolving the material into two adjacent peaks; the use of methanol-water (50:50) on a reversed phase, C–18, semi-preparative column separated the two compounds as white, amorphous powders.

^{1}H nmr spectra of 10-deacetylcephalomannine, (**4**), were remarkably similar to those of cephalomannine (**2**) (9); the methyl region was somewhat better resolved, and the principal difference was the absence of a methyl signal at δ 2.24 attributed to the 10-acetyl group of cephalomannine. In addition, the strong singlet at δ 6.27, characteristic of the isolated methine proton at C–10, had shifted upfield to δ 5.20 due to the absence of the ester group. Eims failed to yield a molecular ion, and the largest fragment (*m/z* 526) was attributed to $C_{43}H_{51}NO_{13}$ (**4**) minus the side chain at C–13, $C_{14}H_{17}NO_4$; smaller fragments were characteristic of the cephalomannine side chain, e.g., *m/z* 55 and *m/z* 83, which are indicative of the tiglic acid moiety (9). The isolated 10-deacetylcephalomannine had tlc and ^{1}H nmr characteristics indistinguishable from those of a reference sample of **4** isolated as a partial hydrolysis product of the parent compound (9).

^{1}H nmr spectra of the isolated 10-deacetyltaxol (**5**) were likewise very similar to those of taxol (**1**) (8, 9), except that the methyl signal at δ 2.20, due to the 10-acetyl group, was absent; an upfield shift of the singlet for the proton at C–10 (δ 6.23 to 5.18) was evident, as was observed earlier with 10-deacetylcephalomannine. Eims gave small fragments, e.g., *m/z* 77 for the loss of the phenyl group characteristic of the taxol side chain (9).

Both 10-deacetylcephalomannine and 10-deacetyltaxol, after rotary vacuum evaporation of the preparative hplc solvent (methanol-water, 1:1), showed contamination from a new, less polar compound (R_f shift from 0.35 to 0.55 in tlc). Preparative tlc permitted isolation of the two less polar compounds. ^{1}H nmr spectra indicated that each compound had the configuration of baccatin V (**8**) rather than that of baccatin III (**6**). Two major differences in spectra were: (a) collapse of the AB quartet at δ 4.2 (associated with the methylene protons at C–20) to an apparent singlet; (b) an upfield shift of the C–7 proton signals from ca. δ 4.2 to a more complex signal at ca. δ 3.6 appearing as a doublet of triplets. The eims were essentially identical to those of the respective parent compounds. These data permitted the conclusion that the new compounds were 7-epi-10-deacetylcephalomannine, (**9**), and 7-epi-10-deacetyltaxol, (**10**).

The lability of cephalomannine and baccatin III has been previously noted (7). The decrease in chromatographic polarity resulting from epimerization at C–7 probably is due to intramolecular hydrogen bonding between the 7α-hydroxyl and the carbonyl oxygen of the 4α-acetoxy group (11). This hydrogen bonding might provide a driving force for the epimerization. However, we have noted that the transformation is reversible. Upon being stored in solution, each of the 7-epimers was observed by tlc to have partially epimerized back to the parent compound, apparently forming equilibrium mixtures.[5] With longer storage, the

[5]While this paper was under review, it was suggested that this facile epimerization is due to a reversible retro-Aldol reaction involving enolization at C–9, ring opening at the bond between C–7 and C–8, and ketonization at C–7. Reversal of the overall process can then give either of the two possible configurations at C–7.

ester side chains were hydrolyzed from each parent compound, and tlc successfully detected traces of 10-deacetylbaccatin III, (**11**), and 10-deacetylbaccatin V, (**12**), in the partially epimerized mixtures; these two tetrols were previously isolated from the partial hydrolysis of cephalomannine (7, 9). Consequently, the facility of epimerization at C-7 and hydrolysis must be kept in mind when these compounds are handled or formulated.

The new taxane esters had KB activities (ED_{50}, μg/ml) as follows: **4**, 3.1×10^{-2}; **5**, 2.7×10^{-2}; **9**, 4.6×10^{-2}; and **10**, 3.4×10^{-2}. Apparently, the 10-acetyl group and the β-hydroxyl at C-7 are not essential to the cytotoxic activities of cephalomannine and taxol, which are active respectively at 3.8×10^{-3} and 1.2×10^{-3} μg/ml (9).

19-Hydroxybaccatin III (**3**) was inactive *in vivo* in the PS mouse lymphocytic leukemia system at the doses tested (0.26–4.3 mg/kg). 10-Deacetylcephalomannine (**4**) (130% T/C at 16 mg/kg) and 10-deacetyltaxol (**5**) (135% T/C at 16 mg/kg) were both PS active; cephalomannine (**2**) and taxol (**1**) have reported respective PS activities of ca. 162% and 152% at 2.2 mg/kg. The greater water solubilities of these more polar taxane derivatives may help to circumvent *in vivo* toxicities of the parent compounds and could show some clinical advantages over taxol and cephalomannine.

EXPERIMENTAL[6]

PLANT MATERIAL.—Roots, stems and needles of *Taxus wallichiana* Zucc. which were collected near Shillong in the state of Assam, India, in December 1972, were supplied through Dr. R. E. Perdue, Jr., Medicinal Plant Resources Laboratory, U.S. Department of Agriculture; the material was originally designated as *Caphalotaxus manni* Hook. (B–602425, PR 36848). The material was stored at 1° until ground through a Wiley mill for extraction.

EXTRACTION AND PRELIMINARY PURIFICATION.—The large-scale ethanolic percolation and purification of 19 kg of plant material, through petroleum ether and chloroform-water partitionings, were described in detail in an earlier report (9). When the PS- and KB-active chloroform extract was resolved through two silica gel columns and developed with chloroform-methanol fraction F216 (KB, <1 μg/ml; PS, T/C 136% at 150 mg/kg) (7, 9) resulted. F216 (22.7 g) was the starting point for the current fractionation study.

FRACTIONATION OF F216.—A total of 7.559 g of F216 was chromatographed on a 2.5 X 100 cm column of silica gel (131 g) packed in a benzene slurry. The column was developed with 250 ml portions of 0%, 10% and 15% ethyl acetate in benzene, then with 500 ml portions of 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 70%, 80% and 90% ethyl acetate in benzene; a final rinse with 750 ml of 100% methanol completed the development. A total of 72 fractions (100 ml each) were collected and combined on the basis of tlc analysis.

The residue (0.293 g) from fractions 30–32 yielded crystals (0.058 g) of 19-hydroxybaccatin III when dissolved in hot methanol and concentrated to ca. 2 ml. The residue (0.835 g) from fractions 33–36 crystallized upon standing, and rinsing with cold ethyl acetate yielded a new lignan (0.1344 g) to be discussed in a later paper.

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[6]Melting points were determined on a Fisher-Johns apparatus and are uncorrected. Both analytical and preparative tlc was performed on silica gel 60 F_{254} plates, 0.25 mm, (E. Merck), developed (except as noted) in chloroform-methanol (10:1), and charred after spraying with 1% potassium dichromate in 40% sulfuric acid. HiFlosil, 60/200 mesh (Applied Science Labs.), was used for column chromatography. Reversed phase hplc was carried out on a Waters model 6000 instrument using a differential refractometer for the detector, a 7.8 X 300 mm C_{18} μBondapak semi-preparative column, and methanol-water (1:1) as the solvent system (flow rate, 2 ml/min). ^1H and ^{13}C nmr spectra were recorded in deuterochloroform using a Bruker WH–90 instrument with tetramethylsilane as the internal reference (MHz: 90 for ^1H, 22.36 for ^{13}C). Eims spectra (low and high resolution) were obtained with a Kratos MS–30 spectrometer; cims were obtained with a Finnigan 4000 instrument using ammonia as the reagent gas. Fractions and compounds were tested *in vitro* in the Eagles 9KB (KB) human nasopharyngeal carcinoma cell culture system and *in vivo* against the P388 (PS) mouse lymphocytic leukemia, by contractors to the National Cancer Institute, using established protocols (12). Reference samples of baccatin III, baccatin V, cephalomannine, taxol, 10-deacetylcephalomannine, 10-deacetylbaccatin III, 10-deacetylbaccatin V and baccatin III oxidized at C-13 were available (7, 9).

The greatest activity (KB, <1 μg/ml) was associated with fractions 37–44 (2.246 g) and 45–52 (1.272 g). Each of these active fractions was developed separately on a 60 X 2.0 cm column of silica gel (46 g) packed in a chloroform slurry. Development was made with methanol-chloroform mixtures as follows: 250 ml portions of 0% and 1% methanol, then a 500 ml portion of 2% methanol, followed by 250 ml portions of 3%, 4%, 5%, 6% and 10 % methanol in chloroform. With each column, 46 fractions of 50 ml each were collected and combined on the basis of similar tlc patterns. An additional quantity (0.084 g) of the lignan crystallized from fractions 24–26 of the rechromatographed fractions 37–44.

The greatest KB activity (<1 μg/ml) was associated with a single tlc spot at R_f 0.35; after small-scale preparative tlc, the ^1H nmr spectrum of this spot was indicative of a mixture of compounds possessing the side chain esters of both taxol and cephalomannine, since it showed partially developed signals at δ 7.76 (N-Bz) and also at δ 1.70 and 1.75 (vinyl methyl groups—see Table 1). Repetitive semi-preparative hplc was used to isolate and separate these two components from appropriate fractions (ca. 25–33) of the two chloroform-methanol columns. 10-Deacetylcephalomannine (0.254 g) and 10-deacetyltaxol (0.135 g) were then identified as the two active compounds; for **4**, the retention time was ca. 42–50 min and for **5**, 60–72 min.

CHARACTERIZATION OF 19-HYDROXYBACCATIN III.—Compound **3** crystallized from methanol (0.058 g, 0.00091% yield); mp 171–174°; tlc, R_f 0.35; cims (NH_3), *m/z* 620 $(M+NH_4)^+$ and 602 $(M+NH_4-H_2O)^+$; high resolution eims (70 eV), *m/z* 543.2294 (observed) and 543.2230 (calculated) for $C_{29}H_{35}O_{10}$ (M–OAc); low resolution eims (70 eV), *m/z* (%); 543 (0.5, M–OAc), 525 (0.7, M–OAc–H_2O), 421 (0.4), 403 (0.5), 402 (0.6), 211 (0.8), 105 (100, Bz) and 77 (28, Ph); ^1H nmr, see table 1; ^{13}C nmr, see table 2.

CHARACTERIZATION OF 10-DEACTYLCEPHALOMANNINE.—Compound **4** was isolated as a white amorphous powder remaining after evaporation of the hplc solvents (0.254 g, 0.0038% yield); tlc, R_f 0.35; low resolution eims (70 eV), *m/z* (%): 526 (0.1), 404 (2), 389 (4), 344 (3), 329 (3), 314 (2), 200 (8), 188 (35), 149 (23), 133 (18), 122 (37), 106 (25), 105 (93), 91 (23), 83 (100), 77 (51), 55 (60) and 43 (49); Found: $M^+-C_{14}H_{17}NO_4$, 526.2260; calcd for $C_{29}H_{34}O_9$, 526.2202. ^1H nmr, see table 1.

CHARACTERIZATION OF 10-DEACETYLTAXOL.—Compound **5** was also isolated as a white amorphous powder remaining after evaporation of the hplc solvents (0.135 g, 0.0021% yield); tlc R_f 0.35; low resolution eims (70 eV), *m/z* (%): 222 (10), 210 (11), 193 (13), 149 (10), 121 (10), 106 (11), 105 (100), 91 (13), 77 (47), 51 (23) and 43 (30); Found $M^+-C_{16}H_{15}NO_4$, 526.2202; calcd for $C_{29}H_{34}O_9$, 526.2202. ^1H nmr, see table 1.

EPIMERIZATION OF COMPOUNDS **9** AND **10**.—The first hplc resolution of **4** and **5** was achieved in a preliminary experiment; the hplc solvent, methanol-water (1:1), was evaporated under rotary vacuum at 50°. Under these conditions, both **4** and **5** were partially converted into less polar compounds at R_f 0.55. After evaporation of methanol in a subsequent experiment, **4** and **5** were successfully removed from the aqueous solution without epimerization by extraction with methylene chloride. However, stable emulsions would form during the partitioning, necessitating centrifugation. In subsequent experiments, the transformation was minimized by lowering the evaporation temperature to 30°. Furthermore, the epimerization also occurred in nonaqueous solvents such as chloroform upon prolonged standing. Preparative tlc on silica gel resolved the new compounds from their precursors and they were isolated after elution with methylene chloride-methanol (3:1). The two new compounds were then identified as 7-epi-10-deacetylcephalomannine (**9**) and 7-epi-10-deacetyltaxol (**10**), which proved that epimerization at C-7 was the source of the artifacts.

CHARACTERIZATION OF 7-EPI-10-DEACETYLCEPHALOMANNINE.—Compound **9** was isolated by preparative tlc; 38.1 mg of the appropriate mixture was applied to a preparative plate, and **9** was isolated as a white amorphous powder (0.011 g). From the partially epimerized mixture, 0.0221 g of **4** was concurrently isolated; for **9** tlc R_f 0.55; low resolution eims (70 eV), *m/z* (%): 526 (0.1), 404 (0.7), 188 (20), 149 (18), 122 (24), 106 (20), 105 (100), 91 (18), 83 (72), 77 (41), 55 (40) and 43 (46); ^1H nmr, see table 1.

CHARACTERIZATION OF 7-EPI-10-DEACETYLTAXOL.—Compound **10** was also isolated by preparative tlc of 38.5 mg of a mixture of epimers; **10** was obtained as a white amorphous powder (0.0147 g). From the partially epimerized mixture, 0.0207 g of **5** was concurrently isolated; properties for **10** include: tlc, R_f 0.55; low resolution eims (70 eV), *m/z* (%): 526 (0.3), 480 (0.2), 210 (10), 188 (8), 149 (11), 122 (15), 106 (12), 105 (100), 91 (10), 83 (26), 77 (34), 51 (10) and 43 (15); ^1H nmr, see table 1.

IDENTIFICATION OF HYDROLYSIS PRODUCTS OF **4**, **5**, **9** AND **10**.—Chloroform solutions of **4**, **5**, **9** and **10** which had been stored for a period of several days under refrigeration were examined with tlc. Each compound had formed some of its corresponding 7-epimer. In addition, lesser amounts of two hydrolysis products were visualized in each mixture; these were identified by tlc as **11** (R_f 0.25) and **12** (R_f 0.45).

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