Semi-Synthesis of Paclitaxel from Naturally Occurring Glycosidic Precursors

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Paclitaxel, an antitumor drug effective on ovarian and breast carcinomas, is currently being produced both by direct isolation from the bark of *Taxus brevifolia* and by semi-synthesis from a natural precursor, 10-deacetyl baccatin III. Although other potential precursors such as 10-deacetyl paclitaxel-7-xyloside were known since 1984, their conversion to paclitaxel could not be achieved because of the lack of suitable methodology for hydrolyzing the xylose residue, compatible with the stability of the compound. A method is described here using periodate, followed by phenylhydrazine, to effect deglycosidation of 10-deacetyl paclitaxel-7-xyloside to form 10-deacetyl paclitaxel. In addition, by including an intermediate acetylation step before the reaction with phenylhydrazine, "direct" conversion of this xyloside to paclitaxel itself, is described. Because 10-deacetyl paclitaxel-7-xyloside occurs at >0.1% in the bark of *Taxus brevifolia*, its successful hydrolytic conversion to paclitaxel represents an extremely important reaction for the enhanced availability of this drug.

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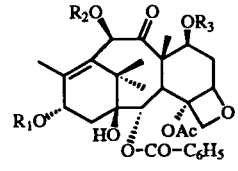
Introduction

Paclitaxel **1**, a taxoid diterpene ester, originally isolated by Wani *et al.*, from the bark of *Taxus brevifolia* [1], has become a well-established antitumor drug for use in the ovarian, breast and other carcinomas [2,3]. Although the bark of the Pacific Yew *(Taxus brevifolia)*, is still the major source, this is being supplemented by two other alternative sources: a) isolation from the needle biomass of *T. x media* Hicksii [4,5], and b) semisynthesis from a natural precursor, 10-deacetyl baccatin III **2** [6], which occurs in the needles of *T. baccata* and several other species. Several elegant methods for this semisynthetic conversion have been published [7-9].

Although details of the large scale process for paclitaxel from the bark of *T. brevifolia* are not known, involvement of two or more chromatographic steps with the "normal phase" silica (or florisil) columns is implied, with an overall yield of 0.01-0.013% [10]. More recently, we described an alternative process applicable to large scale, using "reverse phase" column chromatography on the crude chloroform extract, in which **1** and seven other related taxanes crystallize out directly from the column fractions, with yields of 0.02-0.04% of paclitaxel [11]. Besides this higher yield, this process made readily available, several polar taxane constituents of the bark, such as, 10-deacetyl paclitaxel-7-xyloside **3**, paclitaxel-7-xyloside **4**, 10-deacetyl paclitaxel **5**, 10-deacetyl baccatin III **2**, and others (see Figure 1). The close structural relationship between **3**, **4** and **5**, and **1** suggests that they may also have the potential as precursors for the semi-synthesis of **1**, along with the well-known **2**. In this respect, it must be noted that unlike the case with **2**, the new potential precursors: **3**, **4** and **5** already contain the correct ester side chain at C-13, and thus, their conversion to **1** requires only relatively simpler manipulations such as the removal of the xylose unit and acetylation at **10**.



	R_1	R_2	R_3
1,		Ac	H
2,		H	H
3,		H	xylosyl
4,		Ac	xylosyl
5,		H	H
11,		H	xylosyl
12,		H	H
13,		H	xylosyl
14,		H	H
15,		Ac	H

Figure 1. Paclitaxel and its natural analogues from *Taxus Brevifolia.*

The isolation of the xylosides of paclitaxel and 10-deacetyl paclitaxel was first described by Senilh *et al.*, from the bark of *T. baccata* in 1984 [12]. However, hydrolysis of these to their corresponding aglycones could not be achieved because of the acid-lability of the taxane ring system, and ineffectiveness of enzymatic hydrolysis. For this reason, these xylosides attracted little attention and were regarded as "dead-end analogues" by some researchers in this area.

Although most *O*- and *N*-glycosides are hydrolyzable with acids, the conditions: concentration of the acid, time and temperature may vary over a wide range, depending on the structure [13]. As an unusual example, unlike the readily hydrolyzable adenosine, the closely related pyrrolo[2,3-*d*]pyrimidine riboside (or the 7-deaza) analogue, sangivamycin, was highly resistant to even pro-

longed heating with 6N hydrochloric acid, undergoing instead, hydrolysis of its 7-carboxamide to 7-carboxyl, and the 6-amino to 6-hydroxyl [14]. However, after oxidation of the ribosyl unit with periodate, the hydrolysis proceeded readily in 0.1*N* hydrochloric acid in a short time. The use of periodate as a facilitator of hydrolysis was known since the much earlier studies on polysaccharides, in which the periodate-generated products could be readily hydrolyzed by the action of phenyl hydrazine/acetic acid [15,16]. This method was also applied to the above example of sangivamycin [17]. Based on these findings, the applicability of such facilitated hydrolytic procedure was investigated for the paclitaxel xylosides, and the results are described in this paper.

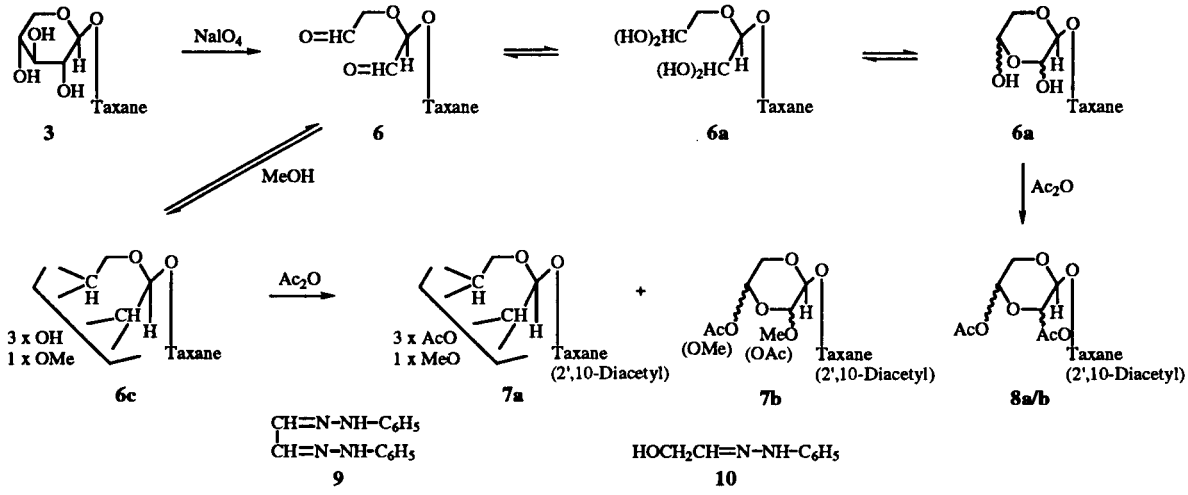


Figure 2. Periodate Oxidation and Acetylation of 10-deacetylpaclitazel-7-xyloside.

Oxidation and Hydrolysis of the Xylosides.

Accordingly, the reaction of 10-deacetyl paclitaxel-7-xyloside **3** with periodate was studied and found to proceed readily, with the consumption of two moles of the reagent to yield a crystalline oxidation product. Thus, the consumption of two moles of periodate confirms the pyranoside structure assigned to the xyloside by Senilh and coworkers [12]. Further more, the α-hydroxy keto system present in **3**, usually regarded as sensitive to periodate, was resistant to oxidation.

Although a dialdehydic compound such as **6** would be expected to result from the periodate oxidation, the nmr spectra (^1H and ^{13}C) of the product showed no aldehyde function. The spectral data indicated that the aldehyde functions of **6** existed as hydrates **6a**, and/or a cyclic "hemialdal" **6b**. Introduction of two new chiral centers in **6b** made the periodate-oxidation product an equilibrium mixture of enantiomers of **6b**, as well as an equilibrium mixture of **6a** and **6b**. In addition, when the oxidation is carried out in methanol, the product reacted with the solvent to form a mixture of methyl acetals **6c**, thereby complicating the picture even further. On acetylation of **6c**, two crystalline products, **7a** and **7b** were isolated. In the case of **7b**, the spectra gave indication for the presence of enantiomeric pairs, as well as for the possibility for the methoxyl being either at 2" or 4" (see Fig. 2). Such behavior shown by the periodate-oxidation products of mono- and disaccharides has been well documented by Guthrie [18]. Acetylation of the periodate-oxidation product, obtained *without* the use of methanol, **6a,b**, also gave a mixture of acetates, of which two could be separated by chromatography and obtained crystalline. Spectral data of these agreed with the structures **8a** and **8b**, which represent a pair of enantiomers. (Table 1).

Acid hydrolysis of **6** in methanol led mostly to bis-dimethyl acetal type products, with some decomposition, and in tetrahydrofuran and 1*N* sulfuric acid (24-48 hours, 25°), 10-deacetyl paclitaxel could be seen as one of the products, but with indications of decomposition. It is noteworthy that the glycosidic bond in this taxane system was significantly resistant to acid even after cleavage by periodate.

The dialdehyde **6** was, however, readily converted to 10-deacetyl paclitaxel **5**, by reaction with phenylhydrazine and acetic acid in methanol. Carbons 1 and 2 of the xylose residue in **6a** generate glyoxal bis phenylhydrazone **9**, followed by the hydrolysis of the original glycosidic bond, and formation of the phenylhydrazone of glycolic aldehyde **10**. Production of the mixed phenylhydrazones can be followed quantitatively by uv-absorbance readings at 370 nm, which showed that the hydrolysis was complete within 1.5-2 hours at 50-60°. At the same time, formation of the 10-deacetyl paclitaxel is also monitored by tlc and/or analytical hplc. The reaction proceeds at

Table 1
NMR Spectra of Compounds **7A/7B** and **8A/8B**

H#	**7a** ppm	**7b** ppm	**8a** ppm	**8b** ppm	C#	**7a** ppm	**7b** ppm	**8a** ppm	**8b** ppm
1	**	**	**	**	1	78.7	78.7	78.9	78.8
2	5.7,d,6.9	5.7,d,6.9	5.7,d,6.9	5.7,d,6.9	2	74.7	74.7	74.5	74.5
3	3.9,d,6.9	3.9,d,6.9	3.9,d,6.9	3.9,d,6.9	3	46.3	45.9	45.9	46
4	**	**	**	**	4	80.6	80.8	80.7	80.7
5	4.9,br,d,8.4	4.9,br,d,8.1	4.9,d,9.6	4.9,d,9.6	5	84	83.9	84	83.9
6a	2.9,m	2.9,m	2.8,ddd	2.8,ddd	6	35.6	35.6	35.8	35.7
6b	2.1,m	2.1,m	2.1,m	2.1,m	*	**	**	**	**
7	4.2,m	4.2,m	4.2,m	4.2,m	7	80.2	80.2	80.2	80.5
8	**	**	**	**	8	57.4	57.4	57.4	57.5
9	**	**	**	**	9	201.3	201.3	201.3	201.3
10	6.4,*s*	6.3,*s*	6.4,*s*	6.4,*s*	10	75	74.8	74.9	74.8
11	**	**	**	**	11	133.3	133.6	133.4	133.4
12	**	**	**	**	12	140.4	140.6	140.7	140.6
13	6.2,br,t,8.7	6.2,br,t,8.4	6.2,t,8.4	6.2,t,8.4	13	71.7	71.7	71.8	71.8
14a	2.4,m	2.4,m	2.3,m	2.4,m	14	35.3	35.3	35.4	35.4
14b	2.2,m	2.2,m	2.2,m	2.2,m	*	**	**	**	**
15	**	**	**	**	15	43.1	43	43.1	43.1
16	1.2,*s*	1.2,*s*	1.2,*s*	1.2,*s*	16	21.3	21.4	21.5	21.5
17	1.2,*s*	1.2,*s*	1.2,*s*	1.2,*s*	17	26.4	26.4	26.4	26.4
18	1.9,br,*s*	1.9,br,*s*	1.8,br,*s*	1.8,br,*s*	18	14.4	14.4	14.5	14.4
19	1.8,*s*	1.8,*s*	1.8,*s*	1.8,*s*	19	10.8	10.7	10.7	10.8
20a	4.3,d,9.1	4.3,d,8.7	4.3,d,8.4	4.3,d,8.4	20	76.4	76.4	76.6	76.6
20b	4.2,d,9.1	4.2,dd,9,3.3	4.2,d,8.4	4.2,d,8.4	*	**	**	**	**
1'	**	**	**	**	1'	169.8	169.8	169.9	169.9
2'	5.5,d,3.3	5.5,d,3.0	5.5,d,3.3	5.5,d,3.3	2'	73.8	73.8	73.9	73.9
3'	5.9,dd,9,3.3	5.9,dd,9.3,3	5.9,dd,9,3.3	5.9,dd,9,3.3	3'	52.8	52.7	52.8	52.7
NH	6.9,d,9.3	6.9,d,9.3	6.9,d,9	6.9,d,9	*	**	**	**	**
1"	4.8,br,*s*	4.4,br,*s*	4.7,br,*s*	4.8,br,*s*	1"	98.8	94.1	95.9	96.5
2H	5.4,br,*s*	4.6,br,*s*	5.6,br,*s*	5.5,br,*s*	2"	96.8	96.8	90.2	86.8
4"	4.5,d,1.5	6.2,dd,9,3	6.1,dd,9,3	5.9,br,*s*	4"	84.8	87.2	84.8	86.7
5"eq	4.0,dd,12,1.5	3.5,dd,9,6	3.6,dd,11,3	3.6,d,12,6	5"	59.8	59.9	59.7	58.8
5"ax	4.2,d,9	4.2,dd,9,3	3.8,t,10.8	4.1,dd,12,1					
OMe	3.53,*s*	3.4,*s*	**	**	OMe	55.3	55.2	**	**

room temperature also, and was complete in about 12-24 hours. At the end of the reaction, **5** is readily isolated by fractional solvent partition and/or column chromatography, and obtained as a crystalline solid.

By using the same procedure, 10-deacetylcephalomannine-7-xyloside **11** was converted to 10-deacetylcephalomannine **12**, and 10-deacetylpaclitaxel-C-7-xyloside **13** to 10-deacetylpaclitaxel-C **14**. Likewise, paclitaxel-7-xyloside (**4**) gave paclitaxel (**1**).

Table 2
Phenylhydrazine-Mediated Hydrolysis

a) **7a/b** (60°) Time (Hours)	Absorbance (370 nm)	b) **6a/b** (25°) Time (Hours)	Absorbance (370 nm)
0	100	0	25
0.5	1600	0.5	370
1	1625	1	465
1.5	1750	1.5	540
3	1750	2	630
		5	660
		23	790
		27	780

Thus, the method described here can be of general utility in cases where deglycosidation by conventional acid hydrolysis may not be applicable, because of the acid sensitivity of the aglycone moiety.

Conversion of 10-deacetylpaclitaxel **5** to **1** was described recently [19], which consisted of protecting the 2' and 7 hydroxyls by either trimethylsilyl or monochloroacetyl groups, acetylation of the 10-OH, and then deprotection. By the use of the former protecting group, an 85% yield for the conversion of **5** to **1** was realized.

However, instead of converting 10-deacetylpaclitaxel-7-xyloside **3** into **5** and subsequently to **1**, a direct conversion of **3** to **1** would be more desirable, and this is also carried out here successfully. For this, the periodate-oxidation product **6** was acetylated to produce the acetate **8**, in which the hydroxyls at 2', 10, as well as those on the recyclized dialdehyde bishydrate, were all acetylated. Reaction of this acetate with phenylhydrazine and acetic acid in methanol yielded paclitaxel-2'-acetate **15** as the major product, with some paclitaxel also being formed during the reaction. Without separation, this mixture of

15 and 1 was subjected to mild basic hydrolysis by methanolic dimethylamine, to effect a smooth conversion to paclitaxel.

In practice, however, most of the steps outlined above can be combined into one composite step and carried out as a "one pot operation", and in the time frame indicated below. Starting with 3, after the periodate oxidation (3 hours), product 6 is recovered (dichloromethane extraction) and acetylated (acetic anhydride and pyridine, 3 hours at 25°). The reagent is decomposed (1:1 methanol/water, 30 minutes), and without isolating the product 8, the mixture is treated with phenylhydrazine at 60° for 3 hours. After extraction with dichloromethane at *p*H 2 (to remove the pyridine and the phenylhydrazine), the product is subjected to the final hydrolysis (dimethylamine in methanol, 1 hour at 25°). Column chromatography of the reaction mixture (C-8 reverse phase) yields paclitaxel. Thus, starting with 6, the steps can all be carried out without the need for the isolation of any of the intermediates.

The first two steps, periodate oxidation and acetylation are essentially quantitative.

In the next two hydrolytic steps, competing reactions such as hydrolysis of the C-2' acetate during the phenylhydrazine reaction, and that of the C-10 acetate during the hydrolysis of the 2'-acetate by dimethylamine, are possible. The former does not pose any problem, and the extent of the latter can be minimized by close monitoring by tlc. Although further optimization may be studied, an overall yield of 50% is commonly observed.

The paclitaxel so obtained was tested for its activity in the cell culture assay using L-1210 cells and found to be equal in activity to that of an authentic sample of paclitaxel. A thorough examination of spectral and other physical properties also confirmed that it was identical with paclitaxel.

Yield Considerations of Paclitaxel.

Although originally isolated from *T. baccata* in 1984 [12], 10-deacetylpaclitaxel-7-xyloside 3 was not recognized as being present in the bark of *T. brevifolia,* occurring to the extent of 0.1+%, until relatively recently [20]. Thus, it did not receive much recognition in the overall strategy of paclitaxel procurement efforts because 1) the isolation methods mostly in use are based on normal phase silica chromatography, and they do not give good separation of this polar taxane from the other polar constituents 2, 11, 13 and others, and 2) methods for the hydrolytic removal of the xyloside residue were also not available. Reverse phase column chromatography can readily yield 3 in pure form, separating it from xylosides 11 and 13, as well as from 2 [20,11]. As shown here, 3 can be readily converted under mild conditions to either 5 or 1, depending on the reaction method used [21,22]. Thus, this reaction serves a much needed purpose of making these glycosides useful in the production of paclitaxel and related compounds. Although the bark of *T. brevifolia* may serve less and less as a source for paclitaxel in future, the interest in the xylosidic analogues will continue, as they are being identified increasingly in the needles of several *Taxus sp.* and among the cell-culture generated taxanes [23,24].

In view of the presently described conversion of the xylosides to paclitaxel, the total yield of paclitaxel realizable from the bark of *T. brevifolia* may be reassessed. As processed by conventional silica column chromatography, the yield of paclitaxel from the bark of *Taxus brevifolia* is generally reported to be between 0.01-0.013% [10], with no significant yields of any other useful analogues being isolated at the same time [25,26]. It is because of such low yields of 1 and of any other useful analogues recoverable from the bark, that pursuing other alternative sources became imperative. However, through the use of the reverse phase column chromatography, yields of 0.02-0.04% of 1, along with 3 (0.1%), 4 (0.008%), 5 (0.008%), and 2 (0.02%) are reported, all on a pilot plant scale [11]. Through the application of the presently described conversion of the xylosidic precursors: 3 and 4 to 1, and that of 5 to 1 as given in [19], one can generate an additional 0.05-0.06% of paclitaxel. Likewise, conversion of 2 into 1, by the well-known procedures [6-9] can produce another 0.01% of paclitaxel. By combining the paclitaxel from all of these, the total yield from the bark of *T. brevifolia* is raised to 0.08-0.11%, nearly an 8-10 fold increase over the current yield of 0.01-0.013% from the bark [10]. Thus this possibility makes the bark of *T. brevifolia* still the best source for paclitaxel, although belatedly.

EXPERIMENTAL

General.

Melting points were determined on a Fisher-Johns hot stage apparatus and are uncorrected. The following instrumentation was used for the spectra recorded here: uv, Perkin Elmer λ3B; ir, Perkin Elmer PE-1420; and nmr, VXR-300, Varian Gemini-300 and General Electric QE-300 spectrometers. Mass spectra (FAB) were obtained on a Finnegan Mat 95Q spectrometer using a cesium gun operated at 15 Kev of energy. Elemental analyses were obtained from the Chemistry Department, University of Florida. Analytical hplc was carried out using Waters 501 pump, with a U6K injector, a 486 tunable absorbance detector and a Goertz Servogor 120 recorder.

Column chromatography (normal phase) was carried out using silica gel (Fisher 100-200 mesh). Reverse phase chromatography was carried out using C-18, or C-8 bonded silica gel (Spherisorb, 15-35 micron diameter, Phase Separations Inc., Norwalk, CT) using acetonitrile/ water mixtures. Thin layer

chromatography was conducted using silica gel (Merck H60-P254/366) (EM Science, Fisher), using dichloromethane containing acetone and/or methanol. Visualization was by uv and spray with 1*N* sulfuric acid followed by charring. For the analytical hplc, 1:1 acetonitrile/water was used at a flow rate of 0.5 ml per minute.

10-Deacetylpaclitaxel-7-xyloside **3**.

This was prepared as described earlier [11] by the use of reverse phase column chromatography on the chloroform extract of the bark of *Taxus brevifolia*. The crystalline solid of **3** which directly separated from the fractions was filtered and recrystallized once from acetone and then from a mixture of methanol and dichloromethane using charcoal, to obtain **3** as a colorless crystalline solid, mp 245-248°, yield 0.1-0.12%.

Periodate Oxidation of **3** to Form **6c**.

To a solution of **3** (1 g, 1.05 m*M*) in a mixture of methanol, dichloromethane and water (25, 25, 5 ml) were added 1*N* sulfuric acid (5 ml) and sodium periodate (0.71 g, 3.3 m*M*). After 3 hours, when tlc showed the absence of **3**, the mixture was diluted with water (100 ml), extracted with dichloromethane (3 x 50 ml) and the extract concentrated to dryness to yield 0.95 g of a white powder.

For purification, the above sample was dissolved in 3:7 acetonitrile/water (15 ml) and applied to a column of C-18 bonded silica gel (30 g) in the same solvent mixture. Elution with a step gradient of 30-60% acetonitrile/water gave the compound in 45-50% solvent eluate. After standing for a week, the crystals that separated out were filtered and dried, yield, 0.6 g; mp 190-193° with softening at 178°; [α]D -42.3° and changing to -35.2° in 12 hours and to 33.1° in 48 hours. In spite of the sample being crystalline, the nmr spectrum showed that it was a mixture, which was difficult to separate, and the sample was not analyzed, but characterized as the acetate (see below).

Acetylation of **6c** to **7a** and **7b**.

A mixture of **6** (1 g), acetic anhydride (5 ml) and pyridine (1 ml) was heated at 80° for 30 minutes. After cooling, water was added and the solid filtered and applied to a column of normal phase silica (35 g) in dichloromethane. Elution with 2% acetone in dichloromethane gave the two components **7a** and **7b**, which were crystallized from acetone/ligroin to obtain as colorless crystalline solids.

The yield of **7a** was 0.45 g, mp 192-194°; [α]D (c, 0.99 $CHCl_3$) -61.4°.

Anal. Calcd. for $C_{60}H_{69}NO_{23}$: C, 61.48, H, 5.93, N, 1.19. Found: C, 61.71; H, 5.98; N, 1.20.

The yield of **7b** was 0.3 g, mp 188-190°; [α]D (c, 0.975, $CHCl)_3$ -83.6°.

Anal. Calcd. for $C_{56}H_{63}NO_{20}$•1/2 H_2O: 62.33; H, 5.98 N, 1.30. Found: C, 62.27; H, 6.10; N, 1.24.

Preparation of **8a/8b**.

To a solution of **3** (3 g) in dimethylformamide (25 ml) was added sodium periodate (2 g) in 15 ml of water and 5 ml of 1*N* sulfuric acid. After 3 hours, when tlc showed reaction to be complete, water was added and the precipitate (**6a,b**) filtered and washed with water. After air-drying, the solid (3 g) was reacted with acetic anhydride (10 ml) and pyridine (2 ml). After 3 hours, tlc showed that the acetylation was complete, at which time, water was added and the solid was filtered and washed with water. It was purified by chromatography on a silica column (100 g) in benzene. Elution with benzene containing 2-5% acetone gave the various fractions which were combined based on the data from tlc monitoring. Compounds **8a** and **8b** were purified by crystallization from acetone/ligroin.

Compound **8a** was obtained as colorless needles, mp 207-209°, yield 1.2 g.

Anal. Calcd. for $C_{57}H_{63}NO_{21}$•H_2O: 61.34; H, 5.87; N, 1.25. Found: C, 61.38; H, 6.07; N, 1.23.

Compound **8b** was obtained as colorless needles, mp 186-187°, yield 0.4 g.

Anal. Calcd. for $C_{57}H_{63}NO_{21}$•H_2O: 61.34; H, 5.87; N, 1.25. Found: C, 61.28; H, 6.09; N, 1.28.

Hydrolysis of **6** to **5**.

To a solution of **6a,b** (1.02 g, 1 m*M*) in methanol (25 ml) was added phenylhydrazine (0.5 ml) and acetic acid (3 ml) and water (5 ml). An aliquot sample was taken immediately and its uv absorbance read at 370 nm. The flask was connected to a water condenser and kept at 60°. From time to time, aliquots were taken and the uv absorbance at 370 nm was monitored. Samples were also taken to check the tlc, comparing with the starting material and the product. After 3 hours, when the uv absorbance remained constant, the mixture was diluted with water (200 ml), acidified, extracted with dichloromethane (3x) and the extract concentrated to a dark red oil. The concentrate was partitioned in a countercurrent fashion, between 3:2 methanol/water and 4:1 benzene/ligroin as the two phases, and using 3 separatory funnels. The benzene ligroin layers which contained the phenyl hydrazones were separated and concentrated to dryness. The combined methanol/water layer was concentrated partially and extracted with dichloromethane (3x) and the organic layer concentrated to yield the crude **5**. Purification was carried out by the reverse phase column procedure using C-18 bonded silica in 30% acetonitrile in water and elution with a step gradient of 30-60% acetonitrile in water as described earlier [11]. The fractions from which **5** crystallized out were filtered and the solid dried, yield, 0.6 g, mp 192-196°. Its spectral data were identical with those of an authentic sample of 10-deacetylpaclitaxel [11].

Hydrolysis of 10-Deacetylcephalomannine-7-xyloside **11** to 10-Deacetyl cephalomannine **12**.

The above procedure was applied to **11** (1 g) and **12** was isolated, yield 0.55 g. Its spectral data were identical with those given in [12].

Hydrolysis of 10-deacetylpaclitaxel-C-7-xyloside **13** to 10-Deacetylpaclitaxel C **14**.

The same procedure was carried out with **13** (1 g) and **14** was isolated as a crystalline solid, yield 0.5 g; mp 168-170°. Its spectral and chromatographic data were identical with those given in [11].

Hydrolysis of **7** or **8** to Paclitaxel.

A mixture of **7ab** (1 g), or **8ab**, phenylhydrazine (0.5 ml), and acetic acid (2 ml) in methanol (25 ml) was heated at 60° for 3-4 hours. The cooled solution was diluted with water (100 ml), acidified, extracted with dichloromethane (3x) and the extract concentrated to an oil. This was taken up in 30% acetonitrile in water and applied to a column of C-18 bonded silica gel (25 g). Elution with a step gradient of 30-60% acetonitrile was carried out and fractions were collected and tested by tlc and analytical hplc. The earlier and minor component (100 mg) was found to

be identical with paclitaxel. The slower and the major component was found to be paclitaxel-2'-acetate **15** [27], yield 0.4 g.

A solution of **15** (0.4 g) in methanol (5 ml) was treated with methanolic dimethylamine (1%, 5 ml) and the solution let stand at room temperature. The progress of the hydrolysis was followed by tlc and when most of **15** disappeared, with only a trace of 10-deacetylpaclitaxel being present, the mixture was acidified, concentrated to remove the solvent and the product crystallized from acetone/ligroin to yield paclitaxel, yield 0.3 g, mp 218-220°. Its chromatographic and spectral data were identical with those of paclitaxel.

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