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Semisynthesis of Taxol®: an improved procedure for the isolation of 10-deacetylbaccatin III

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From the needles of domestic yew, (*Taxus baccata*), 10-deacetylbaccatin III (10-DAB) can be isolated in quantities of up to 297 mg per kg of fresh needles. Additional quantities of 10-DAB can be obtained from the extract by $NaBH_4$ mediated reductive hydrolysis of baccatin esters. A four-step procedure converts 10-DAB into taxol in 58% overall yield.

Key words: anti-tumor agents, taxol, natural products, semisynthesis.

A naturally occuring diterpenoid Taxol® (Paclitaxel) **2**, isolated from the bark of the Pacific yew (*Taxus brevifolia*),[1] is a highly promising antitumor agent: currently approved for the treatment of refractory ovarian and metastatic breast cancer, taxol is undergoing clinical evaluation against many other indications, notably lung and head-and-neck cancers.[2] The commercial production of taxol initially relied on extraction from the bark of the Pacific yew tree. However, this approach proved unsuitable for long term, large scale production: the bark harvesting destroys the tree and seriously threatens the very slow-growing yew tree population.[3] The complex molecular structure of taxol makes it elusive to an economical total synthesis.[4] The solution to the "taxol supply problem" was found in its semisynthesis. Thus, 10-deacetylbaccatin III (10-DAB) **1**, structurally related to taxol, was isolated from the needles of the European yew (*Taxus baccata)* and further transformed into taxol by a four step procedure (Scheme 1).[5] The needles are renewable, and harvesting does not destroy the tree; therefore the method can provide large quantities of semisynthetic taxol. This approach also gave rise to a novel, non-natural taxoid, Taxotere® (Docetaxel) **3**, a compound comparable to taxol in terms of therapeutic indications and efficiency.[6]

The content of 10-DAB in the needles of European yew has been quite extensively investigated, and is known to be dependent on many parameters, such as cultivar, altitude, season, shade-grown or sun-exposed trees, *etc.* In addition, the method and duration of drying the needles appears to be critical in achieving the

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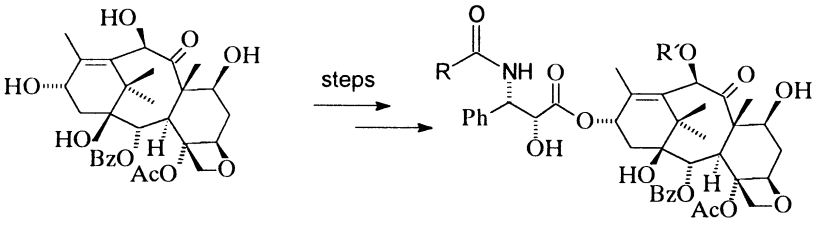


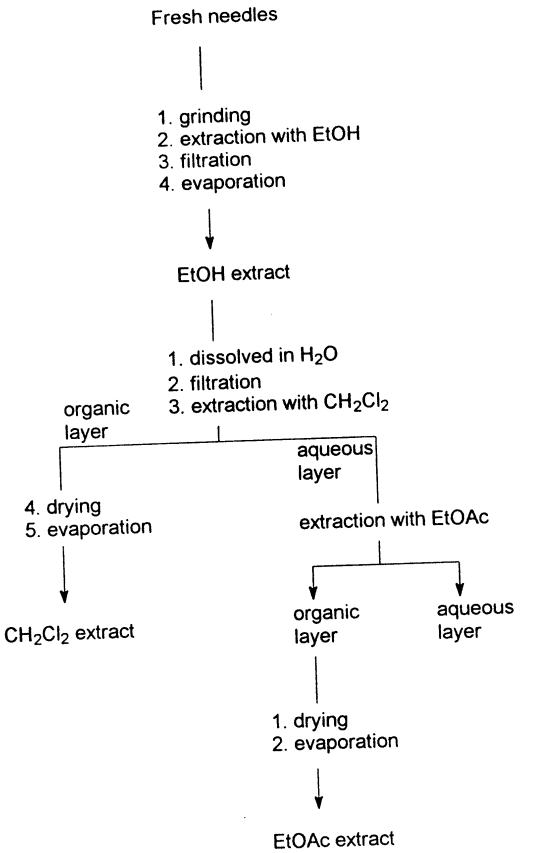
1 10-deacetylbaccatin III

2 R=Ph; R'=Ac: Taxol

3 R=O*t*Bu; R'=H: Taxotere

Scheme 1.

optimum yield of 10-DAB. The reproted contents of 10-DAB varied from 0 to 360 mg/kg of leaves;[7] a notable exception is a report by French researchers, who succeeded in isolating 1 g of 10-DAB per kg of fresh needles.[5] We endeavoured to determine the content of 10-DAB in the needles of *Taxus baccata* originating from the Belgrade area, in various seasons, in order to optimise and further improve the



Scheme 2. Extraction of yew needles.

reported procedure for its isolation and conversion to taxol. Here we wish to report the results of this study.

TABLE I. Isolated yields of 10-deacetyl baccatin III **1** from various needle collections

Entry	Needle collection	Yield of 10-DAB (mg per kg of leaves)		
		CH_2Cl_2-extract	EtOAc-extrct	total yield
1	October 1996[a]	67	b	67
2	October 1997[a]	23	b	23
3	April 1998	31	b	31
4	June 1998	165	41	225
5	July 1998	265	32	297
6	August 1998	97	32	129

a) leaves dried prior to grinding; b) not determined

Six collections of *Taxus baccata* needles were analysed: two spring collections, three summer collections, and one autumn collection.[8] In order to avoid changes in 10-DAB content, which are known to occur on standing or drying, four samples were processed immediately after harvesting, without drying. The fresh needles were suspended in ice-cold ethanol, ground in a blender, and further processed as previously described (see Scheme 2 and the Experimental Section).[7e] In this way two organic extracts were obtained, a CH_2Cl_2-extract and an EtOAc extract. From the former, 10-DAB was isolated by column chromatography, in yields indicated in Table I. Several features are of note:

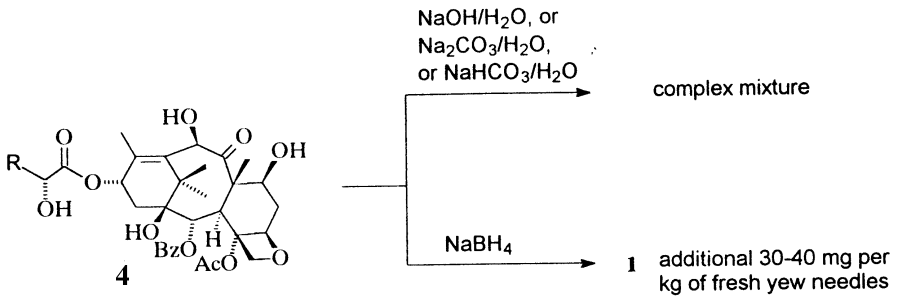
– In contrast to a previous report from this geographic area,[7f] 10-DAB was isolated from all five needle collections, although in very variable yields.

– Much higher yields in 10-DAB were obtained from summer collections than from the spring or autumn ones. In should be pointed out that the two highest yielding collections consisted of only pale-yellow, young leaves. Apparently, these young needles contain much more 10-DAB than old ones (*i.e.,* those remaining from previous vegetative seasons).

– The highest yield of 10-DAB isolated under these conditions was 265 mg per kg of fresh needles. While this yield is lower than that obtained by the French group,[5] it compares favourably to other reports.[7]

In addition to 10-DAB, the extract of *Taxus baccata* needles is known to contain many other taxoids, some of which are essentially derivatives of 10-DAB (*e.g.*, 10-deacetyltaxol, 10-deacetylcephalomanin, *etc.*).[7e] While the individual concentrations of these compounds are too low to allow for an efficient isolation of paclitaxel precursors, we reasoned that their conversion to 10-DAB prior to purification could provide additional quantites of 10-DAB, and improve considerably the overall yield. When fractons of the CH_2Cl_2 extract, not containing 10-DAB, were submitted to hydrolytic conditions, no 10-DAB could be identified in the reaction mixture. However, submitting the EtOAc extract to the same conditions resulted in 10-DAB formation,

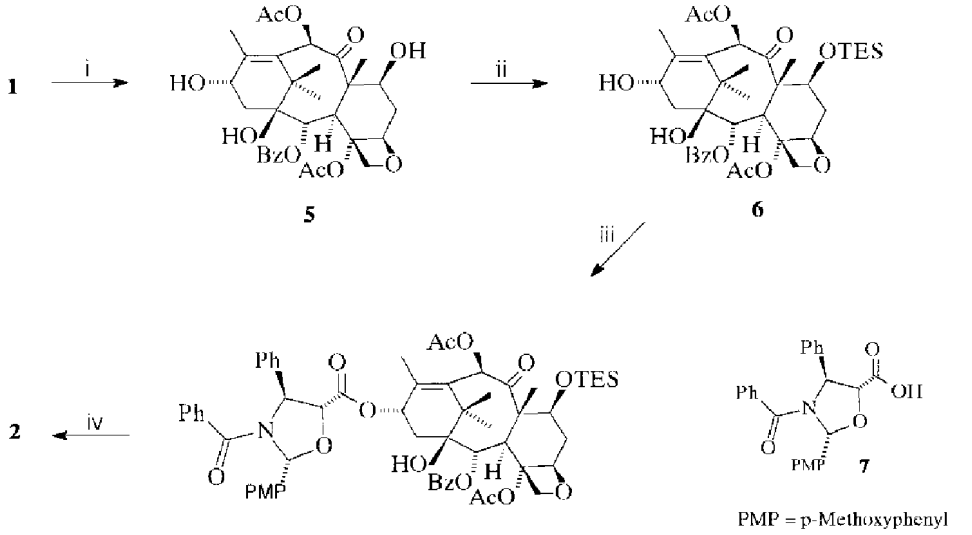
albeit in low yield. The low yield in these reactions can be explained by the lack of regioselectivity in the hydrolysis reactions of baccatin-III derivatives, where, even under mild reaction conditions, many functional groups are affected simultaneously. Therefore, in order to achieve selectivity, we turned our attention toward a special class of 10-DAB derivatives, the α-hydroxy-esters of type **4**, which had been previously identified as constituents of *Taxus baccata* needles extracts, and which could be expected to be hydrolysed regioselectively. As $NaBH_4$ is known to cleave reductively α-hydroxyesters,[9] we hoped that this reagent could perform regioselective hydrolysis of 10-deacetyltaxol, 10-deacetylcephalomanin, and related derivatives, converting them simultaneously into 10-DAB, which is known to be reasonably stable towards $NaBH_4$ under mild conditions. Indeed, treatment of an EtOAc extract with ethanolic $NaBH_4$ resulted in a somewhat better, yet unsatisfactory yield of 10-DAB. Various borohydride based reagents and reaction conditions were tried in order to optimise this conversion. Eventually, we established that $NaBH_4$ reduction in a two phase system (CH_2Cl_2/H_2O) was the method of choice, yielding, after work-up and purification, 30–40 mg of 10-DAB from *ca.* 7 g of EtOAc extract (*i.e.*, per kg of fresh leaves; Scheme 3). In this way, although the exact identity of 10-DAB "precursors" remained unknown, we succeeded to exploit both the CH_2Cl_2- and the so far useless EtOAc extract, and to isolate a total of 297 mg of 20-DAB from 1 kg of fresh yew needles.



Scheme 3.

Conversion of 10-DAB to taxol was achieved in 4 steps, as depicted in Scheme 4. Rather than using the "standard procedure" for the conversion of 10-DAB into suitably protected 7-TES-baccatin III **6**,[5] we used a recently described lanthanide catalysed transformation of 10-DAB into baccatin III **5** (100%),[10] which was further protected as 7-triethylsilyl ether **6** under standard conditions (63%).[5] This order of events gave cleaner reactions and superior yields. DCC mediated coupling of 7-TES-baccatin **6**, with the taxol side-chain protected as aminoacetal **7**, furnished ester **8**, whose double deprotection upon exposure to ethanolic HCl afforded taxol **2**(92% over two steps) identical to the natural product. The overall yield of the 4-step sequence was 58%.

To summarise, we believe to have shown that domestic yew represents a valuable starting material for the semisynthesis of taxol. Our modification of the isolation procedure improves the yield of 10-DAB and makes use of the so far unused fractions of yew needle extract.



Scheme 4.

EXPERIMENTAL

General remarks

NMR spectra were recorded on a Varian/Gemini 200, 200 MHz instrument, using TMS as the reference. Coupling contants are given in hertz.

*Extraction of the plant material and isolation of 10-DAB **1** from the "CH_2Cl_2-extract"*[7e]

Fresh needles (0.6 kg) were finely ground in a blender, under ice-cold ethanol. The resulting suspension was diluted with ethanol (total volume of ethanol: 3 l), and left 48 h with stirring at r.t. The extract was filtered, evaporated under reduced pressure to *ca.* 300 ml, diluted with water to 1 l volume, filtered, and thoroughly extracted with CH_2Cl_2. The organic phase was dried over anhydrous $MgSO_4$, and evaporated under reduced pressure to yield 5.6 g of the "CH_2Cl_2-extract". The aqueous phase was throughly extracted with EtOAc, dried over anhydrous $MgSO_4$, and evaporated under reduced pressure to afford 4.8 g of the "EtOAc-extract".

The CH_2Cl_2-extract was submited to dry-flash chromatography (SiO_2, 45 g, eluent: heptane/EtOAc = 1/1) to afford crude 10-DAB. The crude 10-DAB was submitted to a second dry-flash chromatography (SiO_2, 4 g, eluent: CH_2Cl_2/methanol=95/5), to furnish 111 mg of pure 10-DAB **1**.

^{1}H-NMR (in DMSO): 8.08 (*d*, *J* = 7.8, 2H); 7.7–7.5 (*m*, 3H); 5.4 (*d*, *J* = 6.7, 1H); 5.13 (*d*, *J* = 2.1, 1H); 4.9 (*br.d*, *J* = 9, 1H); 4.6 (*m*, 1H); 4.09 (*m*, 1H); 4.02 (*s*, 2H); 2.28 (*m*, 1H); 2.2 (*s*, 3H); 1.9 (*s*, 3H); 1.65 (*m*, 1H); 1.5 (*s*, 3H); 0.93 (*s*, 6H) and OH-signals at 5.2, 5.0 and 4.8 ppm

*Reductive hydrolysis of baccatin III derivatives **4** and the isolation of 10-DAB **1** from the "EtOAc-extract"*

The EtOAc-extract from the previous step was dissolved in a minimum volume of water (92 ml), and CH_2Cl_2 (92 ml) was added. To this mixture, solid $NaBH_4$ (4.8 g) was added in small portions, with vigorous stirring, over 15 min. After completion of the addition, the reaction mixture was stirred for an additional 2 min, then NH_4Cl (4 g) was added as a concentrated aqueous solution, the layers were separated,

and the aqueous phase extracted twice with CH_2Cl_2. The combined organic extract was dried over anhydrous $MgSO_4$, and evaporated to give 115 mg of the crude product. Purification by dry-flash chromatography (SiO_2, 6 g, eluent: CH_2Cl_2/methanol=95/5) afforded 24 mg of pure 10-DAB **1.**

Baccatin III ***5***[10]

$CeCl_3 \cdot 7H_2O$ (10 mg) was heated to 140 °C, with stirring, under 0.1 mmHg vacuum, for 2.5 h. The resulting powder is allowed to cool to r.t. under an argon atmosphere, then 10-DAB (40 mg) and cold THF (1.5 ml) were added .To this mixture, Ac_2O (170 µl; 10 mol eq) was added, and the reaction mixture was left overnight at r.t. with stirring (the reaction can be monitored by TLC; SiO_2, eluent: heptane/EtOAc = 1/1). The solution was diluted with water, extracted with EtOAc, washed with $NaHCO_3$ and brine, dried over anhydrous $MgSO_4$ and evaporated under reduced pressure to afford 43 mg (quantitative yield) of baccatin III **5**, as a colourless viscous oil that crystallised on standing. This product was used in the next step without purification.

^{1}H-NMR (in $CDCl_3$):8.05 (*dd, J*$_1$=8,*J*$_2$=2, 2H); 7.46 (*m*, 3H); 6.3 (*s*, 1H); 5.58 (*d, J*=7, 1H); 4.94 (*dd*, *J*$_1$=8, J$_2$=2, 1H); 4.82 (*br.t, J* = 9, 1H); 4.42 (*m*, 1H); 4.26 (*d,J*=8, 1H); 4.1 (*d, J*=8, 1H); 3.84 (*d, J*=7, 1H); 2.6 (*m*, 1H); 2.3 (*m*, 2H); 2.24 (*s*,3H); 2.2 (*s*, 3H); 1.98 (*s*, 2H); 1.62 (*s,* 3H); 1.04 (*s*, 6H).

7-Triethylsilyl baccatin ***6***[5]

To a solution of baccatin III **5** (43 mg) in dry pyridine (3 ml), triethylchlorosilane (250 µl) was added with stirring, under an argon atmosphere. After 20 h at r.t., the reaction mixture was partitioned between water and EtOAc, washed with ice-cold dilute HCl, $NaHCO_3$, brine, dried over anhydrous $MgSO_4$, and evaporated under reduced pressure. Purification by dry-flash chromatography (SiO_2, eluent: heptane/EtOAc=7/3) afforded 32 mg (63%) of **6** as white crystals.

^{1}H-NMR (in $CDCl_3$):8.1 (*d,J*=8.5, 2H); 7.6 (*t, J*=8.5, 1H); 7.5 (*t, J*=8.5, 2H); 6.46 (*s*, 1H); 5.63 (*d, J*=7, 1H); 4.95 (*d, J*=10, 1H); 4.83 (*m*, 1H); 4.5 (*dd*, *J*$_1$=11, *J*$_2$=7, 1H); 4.33 (*d,J*=8.5, 1H); 4.16 (*d,J*=8.5, 1H); 3.9 (*d, J*=7, 1H); 2.5 (*m*, 1H); 2.3 (*s*, 3H); 2.19 (*s*, 6H); 1.9 (*m*, 1H); 1.7 (*s*, 3H); 1.19 (*s*, 3H); 1.03 (*s*, 3H); 0.93 (*t,J*=7, 9H); 0.58 (*q, J*=7, 6H).

Taxol ***2***

To a toluene (30 ml) solution of **6** (224 mg), acid **7** (315 mg) was added with stirring, followed by DCC (149.1 mg) and DMAP (45.5 mg). After stirring for 1 h at r.t., the reaction mixture was filtered through a cotton plug, and evaporated under reduced pressure. The residue was taken up in 60 ml of ethanol and cooled to 0 °C. To this solution, ice-cold solution of HCl (1 ml) in EtOH (10 ml) was added, and the mixture was stirred at 0 °C for 44 h (the reaction can be monitored by TLC; SiO_2, eluent: benzene/EtOAc=7/3). The reaction mixture was made alkaline by the addition of aqueous $NaHCO_3$, then excess NH_4Cl_{aq} was added, and the mixture was extracted with CH_2Cl_2. The organic extract was washed with water, dried over anhydrous $MgSO_4$, and evaporated under reduced pressure. Purification of the residue by dry-flash chromatography (SiO_2, eluent: $CHCl_3$/acetone=9/1), followed by recrystallisation from aqueous ethanol afforded 250 mg (92%) of taxol **2**, as a white powder.

^{1}H-NMR (in $CDCl_3$): 8.11 (*d,J*=7.9, 2H); 7.71 (*d*, 7.9, 2H); 7.59 (*t, J* = 7.1, 1H); 7.54 (*m*, 5H); 7.33 (*m*, 5H); 6.97 (*d, J*=8.8, 1H); 6.25 (*s*, 1H); 6.21 (*br.t*, *J*=9, 1H); 5.77 (*dd, J*$_1$=8.8, *J*$_2$=2.6, 1H); 5.65 (*d, J*=7, 1H); 4.92 (*br.d*, *J*=8.9, 1H); 4.77 (*d, J*=2.1, 1H); 4.38 (*dd, J*$_1$=10.6, *J*$_2$=6.8, 1H); 4.28 (*d*, *J*=8.4, 1H); 4.18 (*d*, *J*=8.4, 1H); 3.78 (*d, J*=7, 1H); 3.55 (*br. s*, 1H); 2.53 (*ddd, J*$_1$=15.5, *J*$_2$=9.6, *J*$_3$=6, 1H); 2.43 (*br. s,* 1H); 2.36 (*s,* 3H); 2.34 (*dd, J*$_1$=15.4, *J*$_2$=8.9, 1H); 2.26 (*dd*, *J*$_1$=15.4, *J*$_2$=8.9, 1H); 2.21 (*s*, 3H); 1.86 (*m*, 1H); 1.77 (*s*, 3H); 1.67 (*s*, 3H); 1.22 (*s*, 3H); 1.12 (*s*, 3H).

И З В О Д

СЕМИСИНТЕЗА ТАКСОЛА: ПОБОЉШАН ПОСТУПАК ИЗОЛОВАЊА 10-ДЕАЦЕТИЛБАКАТИНА

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Из лишћа домаће тисе *Taxus baccata*, изолован је 10-деацетилбакатин **1**, у приносима од 23–297 mg/kg свежег лишћа. Нађено је да принос **1** зависи од годишњег доба и старости лишћа, при чему младо, летње лишће садржи највеће количине **1**. Приказана је конверзија 10-деацетилбакатина **1** у таксол **2**.

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