Journal of Labelled Compounds and Radiopharmaceuticals—Vol. XXXVI, No. 8

HEMISYNTHESIS OF [3'-^{14}C]-TAXOTERE®

S. de SUZZONI, C. BOULLAIS, A. VANDAIS, J.P. NOEL*

CEA - CE/Saclay
Service des Molécules Marquées
91191 Gif sur Yvette cedex - France

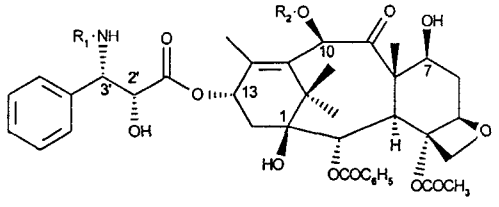
SUMMARY

A new route to semisynthetic Taxotère®, **1**, is described using the mixed anhydride obtained from 2,4,6-trichlorobenzoyl chloride and [3-^{14}C]-cinnamic acid **6**, for the esterification of 7,10-*O*-diTroc-10-deacetylbaccatin III, **4**. Hydroxyamination on the unsatured C-2',3', deprotection of the C-7,10-Troc groups of the ester **7** gave Taxotère® **1**. [3'-^{14}C]-Taxotère® **9** (specific activity : 50 mCi/mmol) was obtained from [3-^{14}C]-cinnamic acid **5'** with a 5% yield.

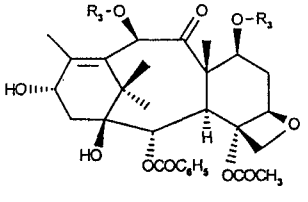
Key words : (^{14}C)-Taxotère, (^{14}C)-cinnamic acid

INTRODUCTION

Taxotère® (Docetaxel) **1** (Rhône-Poulenc Rorer), related to the natural product Taxol® (Paclitaxel) **2**, belongs to a new class of antitumor drugs able to interfere with the microtubule-tubulin system (1). These two taxoids are now well-established as clinically active anticancer agents (2). Paclitaxel can be extracted from yew bark (3), while Docetaxel is prepared by partial synthesis from 10-deacetylbaccatin III, **3** obtained from yew needles (4).



1, R_1= t-BuOCO, R_2=H (Docetaxel)
2, R_1=C_6H_5CO, R_2=Ac (Paclitaxel)



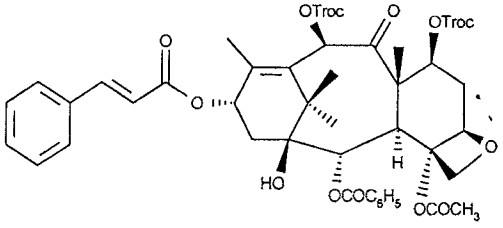
3, R_3 = H(10-deacetylbaccatin III)
4, R_3 = 2,2,2-trichloroethyloxycarbonyl

CCC 0362–4803/95/080739–05

Received 30 November 1994
Revised 2 March 1995

RESULTS AND DISCUSSION

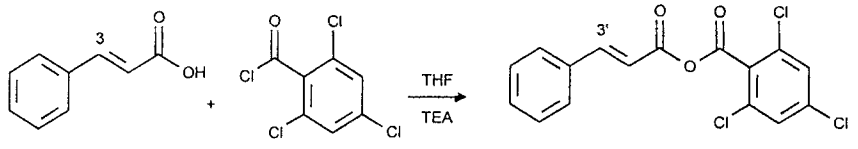
Taxol® N3'-^{14}C was synthesized recently from [7-^{14}C] benzoylchloride and 7-triethylsilylbaccatin III (5). [3'-^{14}C]-Taxotère® labelled on the side chain was required by Rhône-Poulenc Rorer for metabolic studies. The first synthesis was done in 1990. At this time, the semisynthetic route involved the preparation of the cinnamoyl ester 7 from cinnamic acid and baccatin 4, followed by hydroxyamination and deprotection (6). Due to the large excess of cinnamic acid used in the cold syntheses, the preparation of the cinnamoyl ester was the limiting step for our ^{14}C labelled preparation.



7, cinnamoyl ester

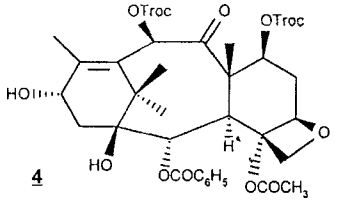
Colin et al. (8) described this reaction of esterification with 5 equiv. of cinnamoyl chloride. They obtained a 55% yield of 7 from the baccatin 4 and a 11% yield from cinnamic acid.

Mangatal et al. (6) described this esterification with 4 equiv. of cinnamic acid activated by DCC/DMAP (Dicyclohexylcarbodiimide//4-dimethylaminopyridine). They obtained a 90% yield from the baccatin 4. Using this method we have obtained the same yield from baccatin 4, but the yield from cinnamic acid was only 15%. The major impurity (50 %) is the "acylurea" arising from the reaction of DCC with cinnamic acid. This by-product is well-known in the litterature (9). The catalysis by p-toluenesulfonic acid according to (10) was not convenient because baccatin III derivatives are very resistant to esterification at C-13 (11); however, with this method, we lost 50 % of the radioactive compound.



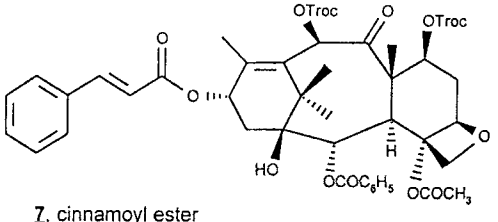
5, cinnamic acid
5', C-3 = ^{14}C

6, mixed anhydride
6', C-3' = ^{14}C



4

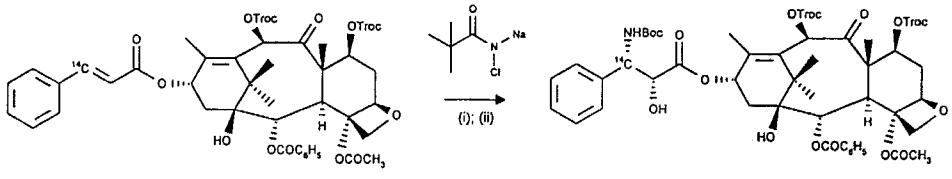
DMAP, toluene



7, cinnamoyl ester
7', C-3' = ^{14}C

After we had observed the good reactivity of cinnamic anhydride with baccatin 4 in presence of DMAP we tried with success the Inanaga's method (12) which recommends to activate acids with 2,4,6-trichlorobenzoyl chloride to form the mixed anhydrides such as 6. The electron withdrawing effect of the trichlorobenzoyl group orients the nucleophilic reaction of the mixed anhydride and baccatin 4. We obtained exclusively the cinnamoyl ester 7 in presence of DMAP or 4-pyrrolidinopyridine with equimolecular stoichiometry of cinnamic acid 5 and baccatin 4, with a 62% yield of isolated ester 7 from cinnamic acid 5. The reaction product was cleaner than that obtained by the DCC/DMAP method and the isolation of the ester was facilitated.This method was patented (13).

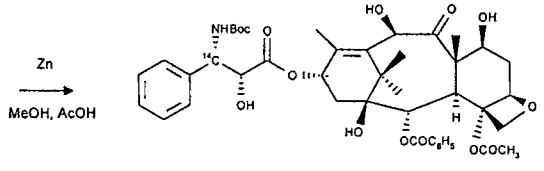
Applied to the reaction of [3-^{14}C] cinnamic acid 5', this method lead to the same yield of [3'-^{14}C] ester 7'. The reaction of hydroxyamination was run according to (6), on the unsaturated C-2',3'. The four diastereomers formed were separated by liquid chromatography and we obtained the hydroxyaminated ester (2'R, 3'S) 8 with a 11% yield, in accordance with literature (6). Cleavage of the 7,10-Troc groups of the labelled ester was obtained with a 75% yield in accordance with the literature (6). This radioactive preparation of [3'-^{14}C] Taxotère® 9 was done twice since 1990 with reproducible results.



7', 3'-^{14}C cinnamoyl ester

(i) $AgNO_3$, OsO_4, CH_3CN
(ii) liquid chromathography

8, 3'-^{14}C hydroxyaminated ester



9, 3'-^{14}C Taxotère^{R}

Materials and methods :

Barium carbonate, specific ativity 56 mCi/mmol, was obtained from Nordion International Inc. All reagents and authentic samples were purchased from Fluka (analytical grade). Baccatin 4 was a gift of Rhône-Poulenc Rorer.
Solvents were dried on molecular sieves.
Radioactive TLC were recorded on a Berthold system, model LB 511.
HPLC were done on Zorbax column from Dupont. The HPLC system was a Merck L 6200. The UV detector was a LKB 2140. The HPLC radioactive monitor was a Berthold, model LB 503.
^{1}H NMR spectra were recorded at 300 MHz on a Bruker AM 400.

EXPERIMENTAL

[3-^{14}C]-cinnamic acid 5' :

Following (7) we obtained 700 mCi of [3-^{14}C]-cinnamic acid from 1000 mCi of $Ba^{14}CO_3$, specific activity : 56 mCi/mmol.

[7,10-O-diTroc-10-deacetylbaccatin III]-13-[3'-^{14}C]-cinnamoyl-ester 7' :

To [3-^{14}C]-cinnamic acid (605 mg # 4 mmol # 210 mCi) in 10 mL of anhydrous THF were added 580 µL of triethylamine. 2,4,6-Trichlorobenzoyl chloride (640 µL # 4 mmol) in 10 mL of anhydrous THF was added dropwise under argon atmosphere with stirring at room temperature. After one hour at room temperature, the triethylamine hydrochloride was filtered off. The THF solution was evaporated under reduced pressure and the mixed anhydride was dissolved in 50 mL of anhydrous toluene. The yield of anhydride was quantitative.

In a three-necked flask flushed with argon, baccatin 4 (4.02 g # 4.4 mmol) and 4-pyrrolidinopyridine (1.16 g # 7.8 mmol) were dissolved in 50 mL of anhydrous toluene by heating at 80 °C. The mixed anhydride in toluene was then added dropwise. TLC monitoring (Silica gel ; toluene-diethyl ether 80:20) showed a 70 % amount of ester 7' after 3 hours at 80 °C.

The organic layer was washed with 1N HCl, $NaHCO_3$ (2.5 %), dried over Na_2SO_4, concentrated under reduced pressure and purified on a silica gel column (200 g Si 60 15-25 µm) eluted by toluene-diethyl ether (95:5) under low pressure (4 bars).

130 mCi (2.5 g # 2.5 mmol) of ester 7' were obtained with a 62 % yield. HPLC analysis was achieved on Silica Zorbax, with an elution by a gradient of isopropanol in heptane (0-5 % in 70 min) rt : 19.30 min. The ^{1}H NMR data of the labelled ester were in agreement with the NMR data of an authentic sample of the non-labelled ester 7 (10 - compound **16**).

[7,10-O-diTroc-10-deacetyl baccatin III]-[3'-^{14}C]-[3'S-(N-tertbutoxycarbonyl)-2'R-(hydroxy)]-13-cinnamate 8 :

To a solution of cinnamate 7' (175 mCi # 3,5 mmol) in acetonitrile (70 mL) were added N-chloro-N-sodio-tert-butylcarbamate (870 mg # 5 mmol), silver nitrate (1,75 g # 10 mmol), a solution (5 mg/mL) of osmium tetroxide in tert-butyl alcohol (3.5 mL) and 0.42 mL of water. The reaction mixture was stirred in darkness at room temperature. After 24 hours, the reaction mixture was filtered. NaCl was added to the filtrate to precipitate Ag^+ into AgCl salt and the reaction mixture was filtered again. Methylene chloride was added and the organic layer was washed with $NaHSO_3$ (2.5 %). HPLC analysis on Silica Zorbax, with an elution by a gradient of isopropanol in heptane (0-5 % in 70 min) showed the following amounts for the products of the reaction : compound 8 : 12.1 % (rt : 42.45 min), for the three other isomers : 7.9 % (rt : 52.25 min) : 7.6 % (rt : 41.30 min), : 8.6 % (rt : 50.20 min). Residual cinnamate 7' was present : 11.8 % (rt : 19.30 min) and dihydroxycompounds (2 isomers) : 15.9 % (rt : 70.30 min) and 17.5 % (rt : 74.20 min). Compound 8 and other isomers were checked by coelution with authentic samples. The organic layer was concentrated and the residue purified by low pressure chromatography on silica gel (200 g Si 60 15-25 µm) eluted by hexane-diethyl ether (1:1). The four diastereoisomers and the residual cinnamate were separated. (2'R, 3'S) hydroxyaminated ester 8 (20 mCi # 0.4 mmol) was isolated with an 11 % yield. The NMR data of the labelled hydroxyaminated ester were in agreement with the NMR data of the unlabelled product (5 - compound **7a**).

[3'-^{14}C]-Taxotere® 9 :

Compound 8 (20 mCi # 0.4 mmol) was treated by zinc dust (460 mg) in 15 mL of methanol-acetic acid (1:1) solution at 60 °C for one hour. In other syntheses the deprotection was also obtained at room temperature for two hours. After filtration, concentration and purification on silica gel column eluted by methylene chloride-ethanol (97:3), [3'-^{14}C]-Taxotere® 9 (15 mCi) was obtained with a 75 % yield. HPLC analysis was done on Zorbax C-8, with an elution by a gradient of methanol in water (55 % to 90 % in 45 min). The purity was 99 % (rt : 29.44 min). TLC (silica gel ; methylene chloride-methanol (95:5)) showed one spot. UV spectrophotometry

analysis in methanol was in agreement with the spectrum of an authentic sample (λ max : 228.7 nm, λ min : 221.7 nm). Specific activity was measured by UV spectrophotometry and liquid scintillation counting. The mass spectrometry analysis (FAB) of [^{14}C-3']-Taxotere® **9** was in agreement with the mass spectrometry analysis of the unlabelled product.

ACKNOWLEDGEMENTS

We thank the Pr. P. Potier group, especially Dr. F. Guéritte-Voegelein, for fruitful discussions and informations about this subject, Rhône-Poulenc Rorer company who ordered this work and for the generous gift of unlabelled compounds, M. H. Virelizier for mass spectrometry analyses and A. Valleix's team (CEA-SMM) for HPLC analyses.

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