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CHEMICAL STUDIES OF 10-DEACETYL BACCATIN III. HEMISYNTHESIS OF TAXOL DERIVATIVES.

F. GUÉRITTE-VOEGELEIN,+ V. SÉNILH,
B. DAVID, D. GUÉNARD and P. POTIER

Institut de Chimie des Substances Naturelles, CNRS,
91190 Gif-sur-Yvette, France

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Abstract - The chemical reactivities of 10-deacetyl baccatin III and of baccatin III, two natural products extracted from Taxus baccata L., were studied with the aim of synthesizing taxol analogues having a modified side-chain at C-13, thereby restoring good binding to tubulin.

In 1971 taxol 1 was isolated from Taxus brevifolia Nutt and was the first taxane diterpene shown to exhibit cytotoxic activity.[1] In vivo, taxol has antileukemic and tumor inhibiting properties[1,2] and it is currently in clinical trials in France and in the USA. The biological activity has been related to the in vitro interaction with microtubule proteins.[3,4] In contrast with other spindle poisons such as vinblastine and colchicine which prevent the assembly of tubulin[5,6] taxol 1 promotes the assembly of microtubules and inhibits the depolymerisation process of tubulin. In addition to taxol, other taxane derivatives showing similar biological activity have been isolated from various species of yew tree.[7-9] Because of its unique mode of action taxol may be the prototype of a new class of chemotherapeutic drugs. However, one of the disadvantages of taxol is associated with its limited availability from natural sources : it is extracted in low yield from the stem bark of the very slow-growing yew tree. To circumvent this major problem several attempts to synthesize the unusual taxane skeleton have been described[10] but to date no total synthesis of taxol has been reported. One other way to prepare this compound is to use simpler taxane derivatives which could be used as precursors in a taxol hemisynthesis.

Baccatin III 2 has been isolated from an alcohol extract of heart wood[8] and 10-deacetyl baccatin III 3 was easily extracted from the annual cut of the yew leaves.[9] These two compounds are not as active as taxol 1 both in vitro and in vivo, but they can be used as raw materials for the preparation of taxol and derivatives. In this paper we wish to report some chemical properties of these compounds and the preparation of new taxane diterpenes which could be used as intermediates in the hemisynthesis of taxol itself.* The compounds obtained in this study have been submitted for in vitro antitubulin evaluation which will allow establishment of structure-activity relationships in this series.

The unusual taxane skeleton of 2 and 3 has a very folded structure (figure 1) in which the α hydroxyl group at C-13 is in a hindered position and furthermore, it can form a hydrogen bond with the 4α acetyl group. It is also important to remember that the 7β hydroxyl can easily epimerize into the 7α isomer via a retro aldol mechanism. The presence of a hydrogen bond with the 4α acetyl group stabilizes the α isomer during the aldol condensation.[11]

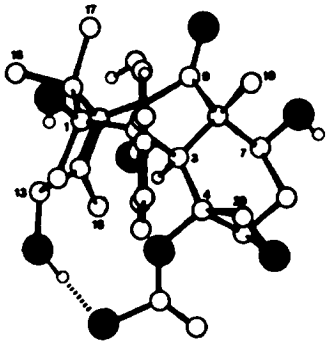


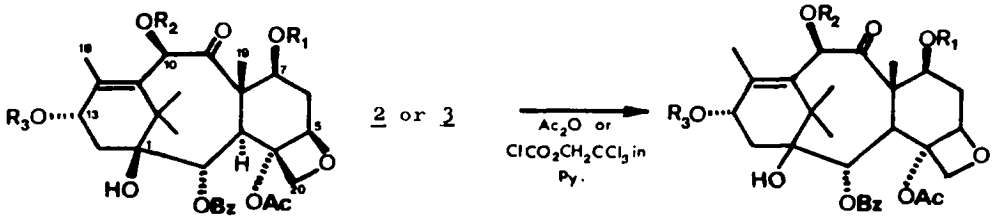
Figure 1.

The other functionalities of tetraol 2 seem stable enough to our experimental conditions, except for the 1-hydroxyl group and the oxetan ring which can be rearranged in acidic media.[12,13]

I. Reactivity of 10-deacetyl baccatin III 3 with acylating agents.

Acetylation of 3 with acetic anhydride yielded three acetylation products depending on the experimental conditions (Table 1). Structure elucidation of these compounds was obtained by considering the chemical shifts of the three protons at C-7, C-10 and C-13 in their proton NMR spectra (see Experimental Part). These data thus show that there is no selectivity between C-7 and C-10 hydroxyl groups toward acylating agents and that the C-13 hydroxyl group is the least reactive, as expected.

Considering these results, we next undertook to protect the two most reactive hydroxyl groups.



1 R_1=H R_2=Ac R_3=COCHOHCHPhNHCOPh
2 R_1=R_2=R_3=H
3 R_1=R_3=H R_2=Ac

4 R_1=Ac R_2=R_3=H
5 R_1=R_2=Ac R_3=H
6 R_1=R_2=R_3=Ac
7 R_1=R_2=CO-O-CH_2-C-Cl_3, R_3=H
8 R_1=$COOCH_2CCl_3$, R_2=Ac, R_3=H
9 R_1=R_2=R_3=$COOCH_2CCl_3$

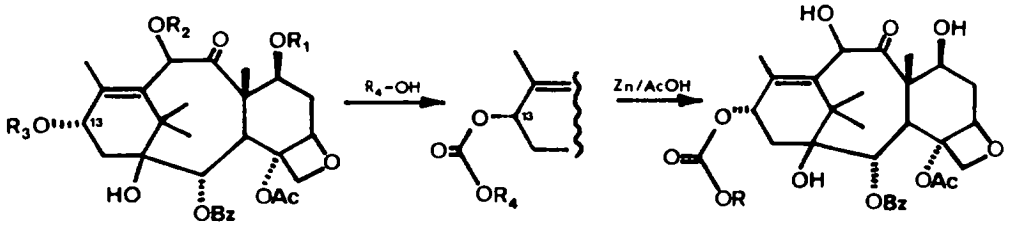
Figure 2.

Table 1.

	Time (h)	Temp.(°C)	4	5	6
1	24	20	48%	48%	-

2. Protection and Deprotection.

Taking into consideration the instability of taxol in basic medium[1] we thought that 2,2,2-trichloroethyl chloroformate could be a good protective group, since it can be removed under very mild conditions. We thus obtained compound 7 in good yield from 10-deacetyl baccatin III 3. With an excess of the acid chloride compound 9 was also prepared. Alkyl 2,2,2-trichloroethyl carbonate can be cleaved by β-elimination with zinc dust in methanol or acetic acid.[15] Compound 7 was cleaved as expected with zinc dust in acetic acid to give the starting material in quantitative yield.



9 $R_1=R_2=R_3=COOCH_2CCl_3$ 10 $R_4=Me$ 11 R=Me
12 $R_4=CH_2-CH_2OH$ 13 $R=CH_2CH_2OH$

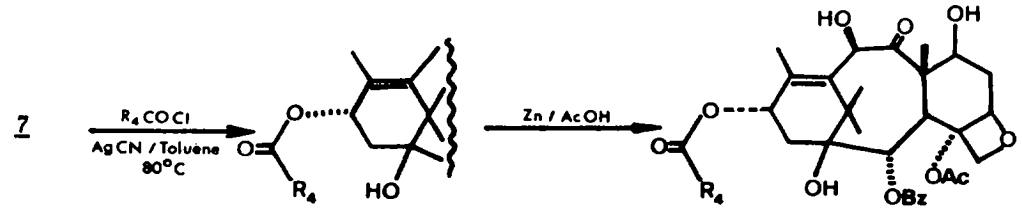
Figure 3.

The same experiments have been done with the 7,10,13-tri(2,2,2-trichloroethyloxycarbonyl)-10-deacetyl baccatin III 9. Treatment of this compound with zinc dust in methanol yielded a new derivative 10. Deprotection of the 7 and 10-positions by reductive cleavage in acetic acid gave a quantitative yield of 13-methyloxycarbonyl-10-deacetyl baccatin III 11. This product was also obtained by direct methanolysis of 9 after deprotection of the 7 and 10-positions. These results led us to try other nucleophilic agents. Thus preparation of compounds 12 and 13 was achieved by treatment of 9 with ethylene glycol. Unfortunately the *in vitro* activities on microtubule assembly of 11 and 13 were less than that of taxol 1.

Baccatin III 3 was protected in the same way to give 8.

3. Hemisynthesis of taxol derivatives from 7 and 8.

Various Taxus species contain a mixture of alkaloids named "taxine".[16] The basic property of these compounds is due to the Winterstein's acid (3-dimethyl amino-3-phenyl propanoic acid). Biosynthetic study of this acid has shown that it arises from phenylalamine by a β-amination of cinnamic acid.[17] It is also well known that cinnamic esters of taxane diterpenes have been isolated from different species of yew trees. Cinnamic acid is thus an attractive candidate for esterification of the free C-13 hydroxyl group of compound 7.



14 $R_4=CH_3$ 15 $R_4=CH_3$

In contrast to the relatively easy acetylation of 7 leading to 14 and 15 after deprotection, acylation with cinnamoyl chloride has proved to be a difficult reaction under the usual experimental conditions. Preparation of 13-cinnamoyl-10-deacetyl baccatin III 17 was finally achieved by the coupling of cinnamoyl chloride and 7 in the presence of silver cyanide at 110°C in toluene, followed by the deprotection of the 10 and 7 positions of 16 by treatment with zinc in acetic acid. The use of more complex acid chlorides such as β phenyl isoserine (Threo and Erythro) to prepare taxol 1 was not successful in our hands. Therefore, our next approach was to investigate some addition reactions on the double bond of the cinnamoyl ester 16.

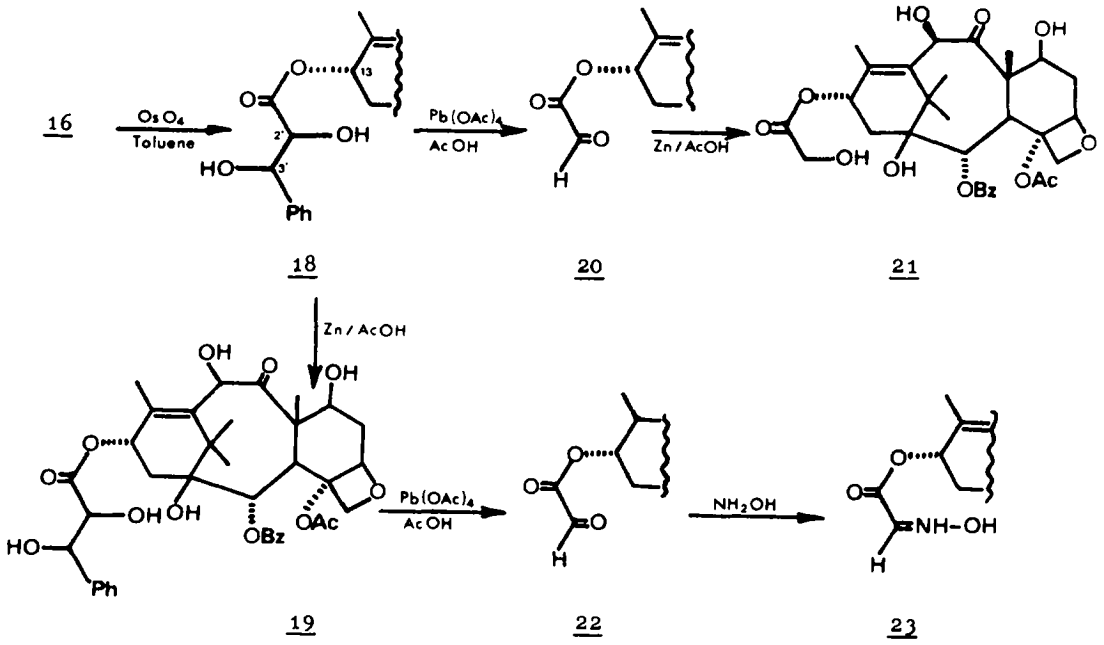


Figure 5.

The relative configuration of the 2' and 3' carbons in taxol and the trans configuration of the cinnamate ester 16 require a cis addition on the 2',3' double bond. The reaction of 16 with osmium tetroxide in pyridine led to the rapid formation of the 2',3'-dihydroxy derivative 18 as a mixture of diastereoisomers (2'S, 3'R and 2'R, 3'S) which could be purified by HPLC of the deprotected mixture 19 (19a and 19b). The ^1H NMR spectrum of 19a and 19b showed two new doublets (J = 2) corresponding to the C-3' and C-2' protons. The FAB mass spectrum gave peaks at m/z 709 (MH^+) corresponding to the addition of two hydroxyl groups.

Oxidation of 18 with lead tetraacetate followed by deprotection of the resulting aldehyde 20 with zinc in acetic acid gave 13-hydroxyacetyl-10-deacetyl baccatin III 21. In a similar manner oxidation of the mixture 19 gave the aldehyde intermediate 22 which was characterized as its oxime 23.

We also tried acylation of baccatin III with crotonyl chloride in order to evaluate the influence of the C-3' phenyl group in 17 on the in vitro activity. Treatment of 7-(2,2,2-trichloroethyloxycarbonyl) baccatin III 8 with crotonyl chloride gave the C-13 ester 24 which yielded 25 after deprotection of the C-7

resulting dihydroxy derivative 26 with zinc in acetic acid gave the ester 27 which is less active on tubulin than the esters 19 containing a phenyl group at C-3'.

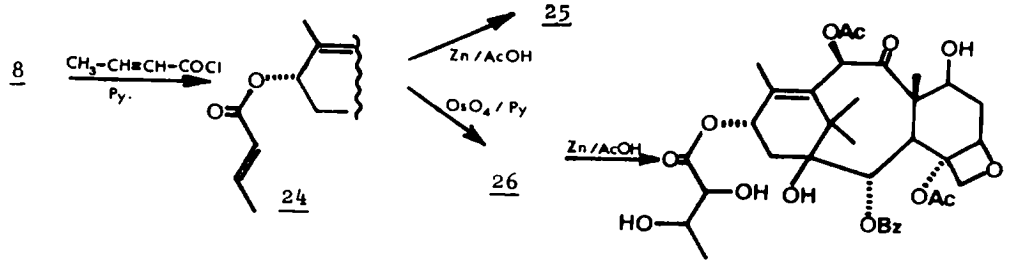


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Figure 6.

Structure-activity relationships of these new taxol derivatives will be discussed in a subsequent publication but it is already interesting to note that, in contrast to the phenyl group, a methyl group at C-3' 25 destroys the in vitro activity and that hydroxyl groups at the 2' and 3' positions 19 increase the activity in comparison to the cinnamate ester 17.

Conclusion.

The results obtained in our work show that it is possible to carry out esterification of the extremely hindered C 13-hydroxy group of baccatin III and 10-deacetyl baccatin III. In particular, hemisynthesis of cinnamate ester 16 allowed us to prepare some taxol derivatives. This compound would seem to be a good precursor of taxol itself using Sharpless hydroxyamination[18] as described previously.[14]

EXPERIMENTAL SECTION

Due to the complexity of the molecules and the small size of the samples available, no elemental analysis is given. Purity of the samples was determined by chromatographic homogeneity and careful analysis of NMR spectra (200 MHz or 400 MHz). Preparative T.L.C. was performed on Merck Silica Gel PF-254 plates. Melting points were observed on a Kofler apparatus, optical rotations measured (c, g/100 ml) on a Perkin-Elmer 141 MC, infrared spectra (νcm^{-1}, $CHCl_3$) on a Perkin-Elmer 257, ultraviolet spectra [EtOH, λmax nm (ε)] on a Jobin-Yvon duospac 203. ^1H NMR spectra were obtained at 200 MHz or at 400 MHz (Bruker AM 200 or AM 400) using TMS as internal standard (coupling constants (J) are given in Hertz (Hz) ; s, d, t, dd and m indicates singlet, doublet, triplet, doublet of doublets and multiplet, respectively). Mass spectra were measured on an AEI MS 9 (CI) or a Kratos MS 80 (FAB). C^{13} NMR Spectra of taxol derivatives will be presented and discussed in a further publication.

Acetylation of 10-Deacetyl baccatin III 2

Exp. 1 : Acetic anhydride (0.1 ml) was added to a solution of 2 (17 mg) in pyridine (1 ml) with stirring at room temperature for 21 h. Work up by standard methods and purification by preparative TLC (solvent : CH_2Cl_2-MeOH, 93-7) yielded 7-acetyl-10-deacetyl baccatin III 4 (10 mg) and 7-acetyl baccatin III 5 (8.4 mg).

Exp. 2 : Acetic anhydride (0.18 ml) was added to a solution of 2 (30 mg) in pyridine (1 ml) with stirring at 60°C for 48 h. Usual work-up and isolation of the products by preparative TLC (solvent : CH_2Cl_2-MeOH, 97-3) gave 5 (17 mg) and 7,13-diacetyl baccatin 6 (18 mg).

Exp. 3 : Acetic anhydride (0.25 ml) was added to a solution of 2 (25.5 mg) in pyridine (1 ml) with stirring at 80°C for 24 h. Usual work up yielded 7,13-diacetyl baccatin III 6 (30 mg).

7-Acetyl-10-deacetyl baccatin III 4

Mp 265-266°C (MeOH-H_2O) ; $[\alpha]_D^{23}$ = -56° (c = 0.53, $CHCl_3$) ; UV : 231(16100), 275(1090), 280(920) ; IR : 3400, 1730, 1710 ; ^1H NMR($CDCl_3$) δ : 1.04 (3H, s, $C_{17}H_3$) 1.09 (3H, s, $C_{16}H_3$), 1.83 (3H, s, $C_{19}H_3$), 1.97 (3H, s, $C_{18}H_3$), 2.08 and 2.32 (2 x 3H, 2s, 2 x OAc), 4.06 (1H, d, J = 7, C_3-H), 4.20 and 4.30 (2H, 2d, J = 9, $C_{20}H_2$), 4.80 (1H, t, J = 9, C_{13}-H), 4.95 (1H, d, J = 9, C_5-H), 5.33 (1H, s,

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