Taxol and Taxotere: Discovery, Chemistry, and Structure–Activity Relationships

DANIEL GUÉNARD, FRANÇOISE GUÉRITTE-VOEGELEIN, AND PIERRE POTIER*

Institut de Chimie des Substances Naturelles, Centre National de la Recherche Scientifique, 91198 Gif-sur-Yvette Cedex, France

Received June 29, 1992

The search for new antitumor compounds using the bioassay-guided fractionation of plant extracts has led to the discovery of a number of novel active structures. Unfortunately, only a few of these substances have proved to be of sufficient pharmacological interest to reach clinical trials. Among these natural compounds, taxol 1,[1] isolated in minute quantities from a number of *Taxus* species, appears today as one of the most promising drugs in the treatment of ovarian and breast cancers.[2]

In addition to its clinical interest, taxol 1 is a powerful tool for biologists interested in the study of the tubulin–microtubule system, proteins which play a crucial role in the construction of the mitotic spindle during cell division[3] and with which taxol interferes.

A third area in which taxol has drawn interest is its partial and total synthesis.[4] In addition to the challenge which the synthesis of this complex molecule presents, the obtention of taxol by synthesis is one of the possible solutions to the serious problem of its limited availability from natural sources.

Reviews concerning the clinical pharmacology,[2] synthesis,[4] biochemistry,[5] and chemistry[6] of taxol have recently appeared. In this complementary Account we summarize the main features concerning the history of the discovery of taxol and report on our own work on the chemistry of the "taxoids" which has led to a new potent antitumor analogue of taxol 1, Taxotere 2. In addition, various structure–activity relationships in the taxoid series will be discussed with a view to clarifying the structural features required for an optimum interaction of taxol-like compounds with their cellular receptor: tubulin.

Daniel Guénard was born in 1946 in Paris, France. He received his Doctorat ès Sciences in 1974 from the University of Orsay and became a staff member of the Centre National de la Recherche Scientifique (CNRS). After a postdoctoral appointment at the Institut Pasteur in Paris, he created the "tubulin laboratory" in the team of P. Potier, involved in the study of the pharmacology and chemistry of spindle poisons. He is interested in the study of molecular conformation and drug–receptor interaction with the help of modeling methods.

Françoise Guéritte-Voegelein was born in 1950 in Rambouillet, France. She received her Doctorat ès Sciences Physique (chimie organique) in 1980 from the University of Orsay. After a postdoctoral appointment at Yale University and Texas A&M University (Prof. A. I. Scott), she joined the team of P. Potier (ICSN, CNRS) at Gif-sur-Yvette and became a staff member of the Institut National de la Recherche Médicale (INSERM). She is interested in the chemistry and structure–activity relationships of new biologically active natural substances.

Pierre Potier was born in Bois-Colombes, France, in 1934. He received his Diploma of Pharmacy (University of Pharmacy of Paris, 1957) and his Ph.D. (Paris, 1960) under the guidance of Profs. Janot and LeMen, and after various stays at the Strathclyde University (Glasgow, U.K.) he joined the Institut de Chimie des Substances Naturelles du CNRS at Gif-sur-Yvette in 1962. He is now the Director of this institute after Janot, Lederer, Sir Derek Barton, and Guy Ourisson. He is Director of Research at the CNRS and has been Professor at the Museum of Natural History (Paris). He is a member of the French Academy of Sciences. His main interests are chemistry (organic and medicinal) and biochemistry.

Taxol: Discovery and Main Features

The pioneering work concerning taxol 1 began in the late 1950s, when the National Cancer Institute (NCI) started a screening program of plant extracts, using tumor system models in vivo and tumor cell lines in vitro. From these studies, the stem bark extract of the Pacific yew tree, *Taxus brevifolia* Nutt., was shown to display cytotoxicity in the KB assay and also activity against carcinosarcoma in rats and leukemia in mice.

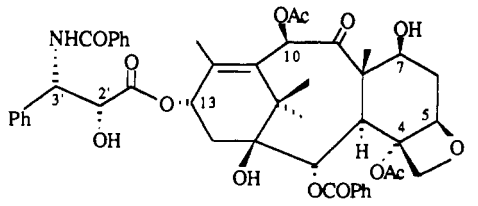
In connection with this NCI screening program, Wall and his collaborators[1] studied the in vitro bioassay guided fractionation of the active extract, and in 1969, taxol was isolated and shown to be the most active constituent of the extract. The structure of taxol was elucidated and published in 1971[1] on the basis of its ^{1}H-NMR spectrum. Methanolysis of taxol led to 10-deacetylbaccatin III **3a** and to the methyl ester of (2*R*,3*S*)-*N*-benzoyl-3-phenylisoserine **4**. Wall assumed that no rearrangement had occurred during this reaction and confirmed the structure and the stereochemistry of taxol 1 by the X-ray analysis of both a derivative of 10-deacetylbaccatin III and a derivative of *N*-benzoyl-3-phenylisoserine methyl ester.[1]

Later, taxol was shown to be a potent inhibitor of cell division with a unique mechanism of action. Horwitz and her collaborators showed that the cellular target of taxol was tubulin.[7] Other natural substances, such as vinblastine, colchicine, and maytansine, were known, at that time, to interact with tubulin by preventing the assembly of tubulin into microtubules. In contrast, taxol acts as a promotor of tubulin assembly as well as an inhibitor of the disassembly process. This new mode of action led to the selection of taxol as a new lead structure for further pharmacological studies.

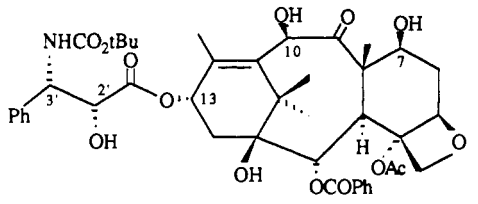
In vivo, taxol shows strong antileukemic and tumor-inhibiting properties with particularly significant activity against the murine B16 melanoma system and against several human tumors.[2] These results opened the way to the clinical investigations of taxol. Phase I studies began in 1983 and showed the important activity of taxol against ovarian cancers. These en-

(1) Wani, M. C.; Taylor, H. L.; Wall, M. E.; Coggon, P.; McPhail, A. T. *J. Am. Chem. Soc.* **1971**, *93*, 2325–2327.
(2) Rowinski, E. K.; Donehower, R. C. *Pharmacol. Ther.* **1991**, *52*, 35–84.
(3) Dustin, P. In *Microtubules*; Springer Verlag: Berlin, 1978.
(4) (a) Blechert, S.; Guénard, D. *Taxus* Alkaloids. In *The Alkaloids, Chemistry and Pharmacology*; Brossi, A., Ed.; Academic Press: San Diego, 1990; Vol. 39, pp 195–238. (b) Swindell, C. S. *Org. Prep. Proced. Int.* **1991**, *23*, 465–543. (c) For recent publications describing total approaches to taxol, see: *Tetrahedron* **1992**, *48* (34).
(5) Horwitz, S. B. *Trends Pharmacol. Sci.* **1992**, *13*, 134–136.
(6) Kingston, D. G. I. *Pharmacol. Ther.* **1991**, *52*, 1–34.
(7) Schiff, P. B.; Fant, J.; Horwitz, S. B. *Nature* **1979**, *277*, 665–667.

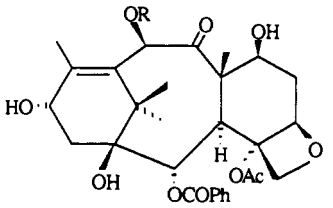
0001-4842/93/0126-0160$04.00/0



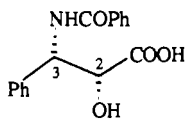
1



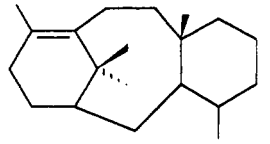
2



3a R=H
3b R=$COCH_3$



4



5

couraging results were confirmed during phase II clinical trials in both advanced and chemoresistant ovarian cancers. Today taxol has entered phase III studies, and this presents a major problem, as these require large amounts of the drug.

Although taxol was initially isolated from the trunk bark of *T. brevifolia* (in a yield of 0.02%), other *Taxus* species from all over the world have since been shown to contain taxol but in limited amounts.[6] A number of other taxol-type diterpenes, recently reviewed by Kingston[6] and one of us (D.G.),[4a] have also been isolated from various species of *Taxus*, but none of these were considered potent enough for therapeutic development. Because 2500 trees need to be harvested to yield 1 kg of taxol,[8] other sources of taxol are being explored in order to meet its growing demand.

Organic chemists have obviously been interested in the total synthesis of taxol 1. Various approaches have been studied in order to build the tricyclopentadecene system 5.[4] In 1988, Holton and collaborators succeeded in the first synthesis of a taxol-related compound, (−)-taxusin (an enantiomer of the natural diterpene).[9] Besides the various strategies for the synthesis of the taxane ring, other studies have focused on the synthesis of the oxetane ring from cyclohexane derivatives[10] and from a simpler natural taxane compound, taxine B.[11]

But to date the most promising approach for the large production of taxol 1 is its partial synthesis from 10-deacetylbaccatin III **3a**, a natural substance isolated from the leaves of the yew tree.[6,12]

Discovery of Taxotere and Partial Synthesis of Taxol

The history of the discovery of Taxotere dates back to 1979 when Horwitz found that the cellular target of taxol was tubulin.[7] At that time, one of the topics of our laboratory was the search for new antimitotic agents belonging to the colchicine, steganacine, and vinblastine series (in other words, the "spindle poisons"). An in vitro bioassay, the tubulin assay described in the next section, was used to evaluate the activities of synthetic or natural compounds. In the vinblastine series, these studies led to the selection of Navelbine, which is now used in the treatment of non-small-cell lung cancer and breast cancer.

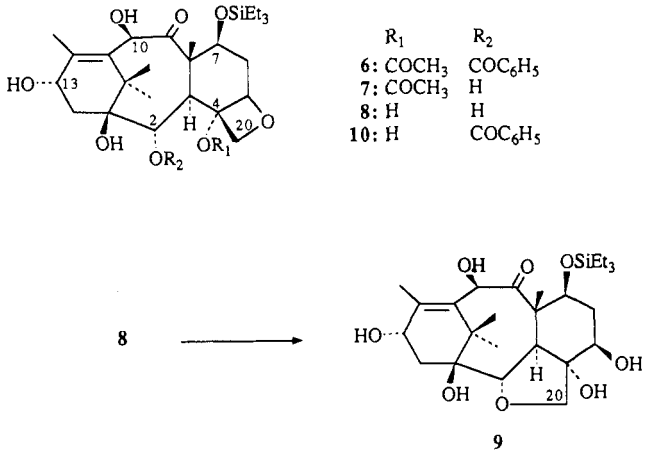
Meanwhile, we had access to some yew trees, *Taxus baccata* L. (European species), which had to be felled to make way for a new road across the campus at Gif. Being aware of the problem already mentioned concerning the scarcity of taxol, we screened various parts (leaves, roots, trunk bark, heartwood) of the yew, using the tubulin assay for monitoring the fractionation of the extracts.

10-Deacetylbaccatin III 3a: Isolation and Chemical Reactivity. Most of the products isolated were shown to possess the tricyclic carbon skeleton bearing the oxetane ring and various hydroxyl and/or ester groups at C-7, C-10, and/or C-13.[12] The main active constituent isolated from the leaves was 10-deacetylbaccatin III **3a** with a yield of 0.02%[12] which could be improved to 0.1%.[13] This compound had previously been found as a degradation product of the methanolysis of taxol[1] and had also been isolated from other species of *Taxus*.[6,14] 10-Deacetylbaccatin III **3a** was of immediate interest to us as it was seen to be an easily and permanently accessible taxol precursor.

The chemistry of taxoids bearing a side chain at C-13 has been studied under mild basic conditions by Wall,[4] McLaughlin,[15] Miller,[16] and Kingston.[17] From these studies it was shown that deacylation occurs at C-10 and/or C-13. Moreover, the 7β hydroxyl group can easily epimerize into the 7α isomer via a retro-aldol mechanism. The reactivity of taxol and its derivatives toward acylation, reduction, and oxidation has been reviewed by Kingston.[6]

(8) Cragg, G. M.; Snader, K. M. *Cancer Cells* **1991**, *3*, 233–235.
(9) Holton, R. A.; Juo, R. A.; Kim, H. B.; Williams, A. D.; Harusawa, S.; Lowenthal, R. E.; Yogai, S. *J. Am. Chem. Soc.* **1988**, *110*, 6558–6560.
(10) (a) Berkowitz, W. F.; Amarasekara, A. S.; Perumattam, J. J. *J. Org. Chem.* **1987**, *52*, 1119–1124. (b) Lin, J.; Nikaido, M. M.; Clark, G. *J. Org. Chem.* **1987**, *52*, 3745–3752. (c) Francl, M. M.; Hansell, G.; Patel, B. P.; Swindell, C. S. *J. Am. Chem. Soc.* **1990**, *112*, 3535–3539. (d) Wender, P. A.; Rawlins, D. B. *Tetrahedron* **1992**, *48*, 7033–7048.
(11) Ettouati, L.; Ahond, A.; Poupat, C.; Potier, P. *Tetrahedron* **1991**, *47*, 9823–9838.
(12) (a) Chauvière, G.; Guénard, D.; Picot, F.; Sénilh, V.; Potier, P. *C. R. Seances Acad. Sci., Ser. 2* **1981**, *293*, 501–503. (b) Sénilh, V.; Blechert, S.; Colin, M.; Guénard, D.; Picot, F.; Potier, P.; Varenne, P. *J. Nat. Prod.* **1984**, *47*, 131–137.
(13) Denis, J.-N.; Greene, A.; Guénard, D.; Guéritte-Voegelein, F.; Mangatal, L.; Potier, P. *J. Am. Chem. Soc.* **1988**, *110*, 5917–5919.
(14) Kingston, D. G. I.; Hawkins, D. R.; Ovington, L. *J. Nat. Prod.* **1982**, *45*, 466–470.
(15) McLaughlin, J. L.; Miller, R. W.; Powell, R. G.; Smith, C. R., Jr. *J. Nat. Prod.* **1981**, *44*, 312–319.
(16) Miller, R. W.; Powell, R. G.; Smith, C. R., Jr.; Arnold, E.; Clardy, J. *J. Org. Chem.* **1981**, *46*, 1469–1474.
(17) Magri, N. F.; Kingston, D. G. I.; Jitrangsri, C.; Piccariello, T. *J. Org. Chem.* **1986**, *51*, 3239–3242.

Scheme I. Hydrolysis of 7-*O*-(Triethylsilyl)-10-deacetylbaccatin III 6



As part of our work on the partial synthesis of taxol, we studied the chemical reactivity of 10-deacetylbaccatin III **3a** under acidic and basic conditions.[18] Alkaline hydrolysis of 7-*O*-(triethylsilyl)-10-deacetylbaccatin III **6** led firstly to the hydrolysis of the benzoyl group at C-2 to give compound **7** and then to the hydrolysis of the acetyl group at C-4 to give **8** (Scheme I). The latter rearranged easily under basic or mild acidic conditions to give the tetrahydrofuran derivative **9**. The opening of the oxetane group under basic conditions was unexpected, but molecular modeling studies on **8** showed that the hydroxyl group at C-2 was very close to the C-20 methylene group of the oxetane, facilitating the intramolecular attack of the C-2 hydroxyl on the oxetane. Using metal hydride, the 4-deacetyl derivative **10** was also obtained from **6**. The fact that compound **10** was only obtained under conditions of metal hydride reduction suggests that the cleavage of the ester group at C-4 occurs by intramolecular hydride attack by a C-13 alkoxy hydride complex. Hydrolysis of the ester groups has also been studied by Kingston[6] and Farina.[19]

Under acidic conditions, the 7,10-diprotected derivative of 10-deacetylbaccatin III **11** led to new compounds with structural modifications on the A ring and on the oxetane (Scheme II). Depending on the experimental conditions, three types of rearrangement occurred. First, a Wagner–Meerwein-type rearrangement occurred on ring A and led to compounds **12** and **13**. Then, opening of the oxetane group takes place to give products **14** and **15**. These types of reactions have previously been observed when taxol has been reacted with electrophilic reagents.[20] Moreover, cyclic ketals **16** and **17** were obtained from compounds **13** and **15** (Scheme II). The reactivity of the C-9 keto group was at first rather surprising, but once again molecular modeling studies supported this and suggested that a hydrogen bond can exist between the hydroxyl of the 2-propanol group and the carbonyl group facilitating an intramolecular attack.

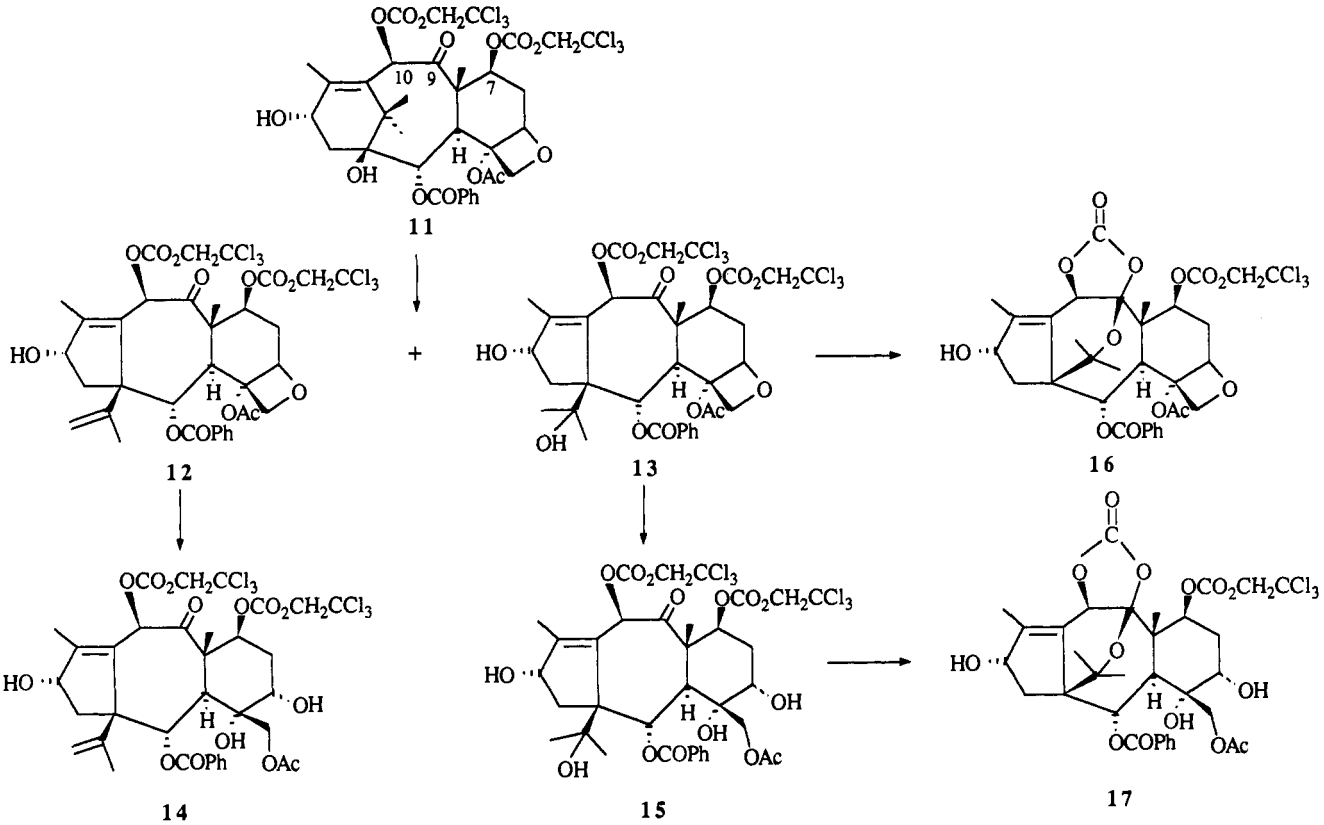
The chemical reactivity of the hydroxyl groups of 10-deacetylbaccatin III **3a**[21] and baccatin III,[17] isolated in low yield from *Taxus* species,[6] and of chemically modified analogues showed that the C-13 hydroxyl group is usually the least easily acetylated. The weak reactivity of the C-13 OH is associated with the steric hindrance at this position due to the presence of the C-4 acetate group; this point is underlined by the fact that the conformation of ring A is modified when the acetate group is removed (compound **10**, Scheme I), as verified by NMR and molecular modeling experiments. In contrast, the opening of the oxetane ring does not bring about any change in the conformation of rings A and B while the C ring adopts a true chain conformation instead of a pseudo chair conformation.

Oxyamination Procedure: Discovery of Taxotere. After suitable protection of the C-7 and C-10 hydroxyl groups of **3a**,[21] our initial studies showed that the C-13 hydroxyl group was difficult to esterify with the acid corresponding to the side chain of taxol. We thus developed a partial synthesis of taxol from the more synthetically available 13-cinnamoyl derivative **18a**[22] and *tert*-butyl *N*-chloro-*N*-sodiocarbamate **19**, using the Sharpless hydroxyamination procedure[23] (Scheme III). The instability of taxol-like compounds to acidic and basic conditions was taken in account in the choice of the *tert*-butyl carbamate derivative **19** as the *t*-BOC group can be removed under very mild conditions. Neither regioselectivity nor diastereoselectivity was observed in the hydroxyamination, and four isomers, **20–23**, were obtained. However, a better stereoselectivity was obtained, using quinine and quinidine derivatives as chiral auxiliaries.[22c] Simple deprotection of the *t*-BOC group followed by benzoylation and removal of the protective groups at C-7 and C-10 afforded 10-deacetyltaxol **24** and the corresponding structural analogues **25–27**. The same reactions when applied to the C-13 cinnamate drivative of baccatin III **18b** led to taxol **1** and its isomers **28–30**. Among the 7,10-diprotected oxyaminated substances, **20** led to compound **2**, after removal of the protecting groups at C-7 and C-10. This substance (RP56976), now referred to as Taxotere, shows a slightly better activity on tubulin than does taxol;[24,25a] consequently Taxotere **2** was selected for further biological studies and proves to possess significant in vitro[25] and in vivo[26] antitumor activities.

Esterification of 10-Deacetylbaccatin III 3 with the Acid Side Chain of Taxol and Taxotere. The oxyamination procedure using osmium tetraoxide is not of practical value for the industrial syntheses of taxol or Taxotere. The direct esterification of 10-deacetylbaccatin III **3a** with the acid corresponding to the side chain of taxol or Taxotere is certainly a superior route. This latter method provided the first direct

(18) Wahl, A.; Guéritte-Voegelein, F.; Guénard, D.; Le Goff, M.-T.; Potier, P. *Tetrahedron* **1992**, *48*, 6965–6974.
(19) Farina, V.; Huang, S. *Tetrahedron Lett.* **1992**, *33*, 3979–3982.
(20) (a) Guéritte-Voegelein, F.; Guénard, D.; Potier, P. *J. Nat. Prod.* **1987**, *50*, 9–18. (b) Samaranayake, G.; Magri, N. F.; Jitangsri, C.; Kingston, D. G. I. *J. Org. Chem.* **1991**, *56*, 5114–5119.
(21) Guéritte-Voegelein, F.; Sénilh, V.; David, B.; Guénard, D.; Potier, P. *Tetrahedron* **1986**, *42*, 4451–4460.
(22) (a) Colin, M.; Guénard, D.; Guéritte-Voegelein, F.; Potier, P. Eur. Pat. Appl. EP 253,738 (Cl.C07D305/14), 20 Jan 1988, FR Appl. 86/10,400, 17 Jul 1986; *Chem. Abstr.* **1988**, *109*, 22762w. (b) Colin, M.; Guénard, D.; Guéritte-Voegelein, F.; Potier, P. Eur. Pat. Appl. EP 253,739 (Cl.C07D305/14), 20 Jan 1988, FR Appl. 86/10,401, 17 Jul 1986; *Chem. Abstr.* **1988**, *109*, 22763x. (c) Mangatal, L.; Adeline, M.-T.; Guénard, D.; Guéritte-Voegelein, F.; Potier, P. *Tetrahedron* **1989**, *45*, 4177–4190.
(23) Herranz, E.; Biller, S. A.; Sharpless, K. B. *J. Am. Chem. Soc.* **1978**, *100*, 3596–3598.
(24) Guéritte-Voegelein, F.; Guénard, D.; Lavelle, F.; Le Goff, M.-T.; Mangatal, L.; Potier, P. *J. Med. Chem.* **1991**, *34*, 992–998.
(25) (a) Ringel, I.; Horwitz, S. B. *J Natl. Cancer Inst.* **1991**, *83*, 288–291. (b) Lavelle, F.; Fizames, C.; Guéritte-Voegelein, F.; Guénard, D.; Potier, P. *Proc. Am. Assoc. Cancer Res.* **1989**, *30*, 2254.
(26) Bissery, M.-C.; Guénard, D.; Guéritte-Voegelein, F.; Lavelle, F. *Cancer Res.* **1991**, *51*, 4845–4852.

Scheme II. Rearrangement of 7,10-Bis-*O*-((β,β,β-trichloroethoxy)carbonyl)-10-deacetylbaccatin III 11 under Acidic Conditions



synthesis of taxol 1 from 10-deacetylbaccatin III **3a** and has been developed in our laboratory, in collaboration with Greene and his collaborators[13] (Scheme IV).

Total synthesis of the acid side chain was first performed by Greene and collaborators.[27] Other strategies with improved yields have also been described.[28] Controlled silylation and acetylation of **3a** led to 7-*O*-$SiEt_3$ baccatin III **31** in good yields.[13] Investigation of the esterification procedure led to the development of a protocol using the 2′-protected acid side chain (6 equiv), di-2-pyridyl carbonate (6 equiv), and 4-(dimethylamino)pyridine (2 equiv). Concomitant removal of the protecting groups at C-2′ and C-7 of compound **32** led to taxol **1**.

In a similar way, Taxotere **2** has been prepared from **3a** and the suitably protected acid side chain,[29] via the triprotected derivative **33**.

Since these first experiments, the yield of the esterification method has been improved by the teams of Holton,[28c] Commerçon,[28h] and Ojima.[28i]

Thus, the problem of supplying the required quantities of taxol, Taxotere, and bioactive structural analogues has been resolved using a semisynthetic process (see above) starting from an easily accessible compound (10-deacetylbaccatin III **3a**) obtained from a natural and renewable source (yew leaves).

Structure–Activity Relationship Studies

A number of new synthetic analogues were prepared[24,30] in order to study the structure–activity relationships in this series. Moreover, X-ray analysis of Taxotere[31] gave, for the first time, the exact conformation of a "taxoid" possessing a side chain at C-13. During our studies, we compared the in vitro activity on tubulin of the various taxol analogues with their conformations determined by NMR experiments and molecular modeling. It must be pointed out that the conformation of taxol has recently been studied by NMR spectroscopy and molecular modeling studies.[32]

Evaluation of the Biological Activity. The determination of structure–activity relationships implies that the biological activity of the products under examination should be evaluated using a very reliable assay. Besides the in vivo assays, measurement of the cytotoxicity on tumor cell lines and monitoring of temperature-induced tubulin–microtubule assembly or disassembly are the most used tools for evaluating the antimitotic activity of the compounds.

(27) Denis, J.-N.; Greene, A.; Serra, A. A.; Luche, M.-J. *J. Org. Chem.* **1986**, *51*, 46–50.

(28) (a) Denis, J.-N.; Correa, A.; Greene, A. *J. Org. Chem.* **1990**, *55*, 1957–1959. (b) Palomo, C.; Arrieta, A.; Cossio, P.; Aizpurua, J. M.; Mielgo, A.; Aurrekoetxea, N. *Tetrahedron Lett.* **1990**, *31*, 6429–6432. (c) Holton, R. A. Eur. Pat. Appl. EP 400,971, 1990; *Chem. Abstr.* **1991**, *114*, 164568q. (d) Honig, H.; Senfer-Wasserthal, P.; Weber, H. *Tetrahedron* **1990**, *46*, 3841. (e) Denis, J.-N.; Correa, A.; Greene, A. *J. Org. Chem.* **1991**, *56*, 6939–6942. (f) Ojima, I.; Habus, I.; Zhao, M.; Georg, G.; Jayasinghe, L. R. *J. Org. Chem.* **1991**, *56*, 1681–1683. (g) Denis, J.-N.; Correa, A.; Greene, A. *J. Org. Chem.* **1991**, *56*, 6939–6942. (h) Commerçon, A.; Bézard, D.; Bernard, F.; Bourzat, J. D. *Tetrahedron Lett.* **1992**, *33*, 5185–5188. (i) Ojima, I.; Habus, I.; Zhao, M.; Zucco, M.; Park, Y. H.; Sun, C. M.; Brigaud, T. *Tetrahedron* **1992**, *48*, 6985–7012. (j) Deng, L.; Jacobsen, E. N. *J. Org. Chem.* **1992**, *57*, 4320–4323.

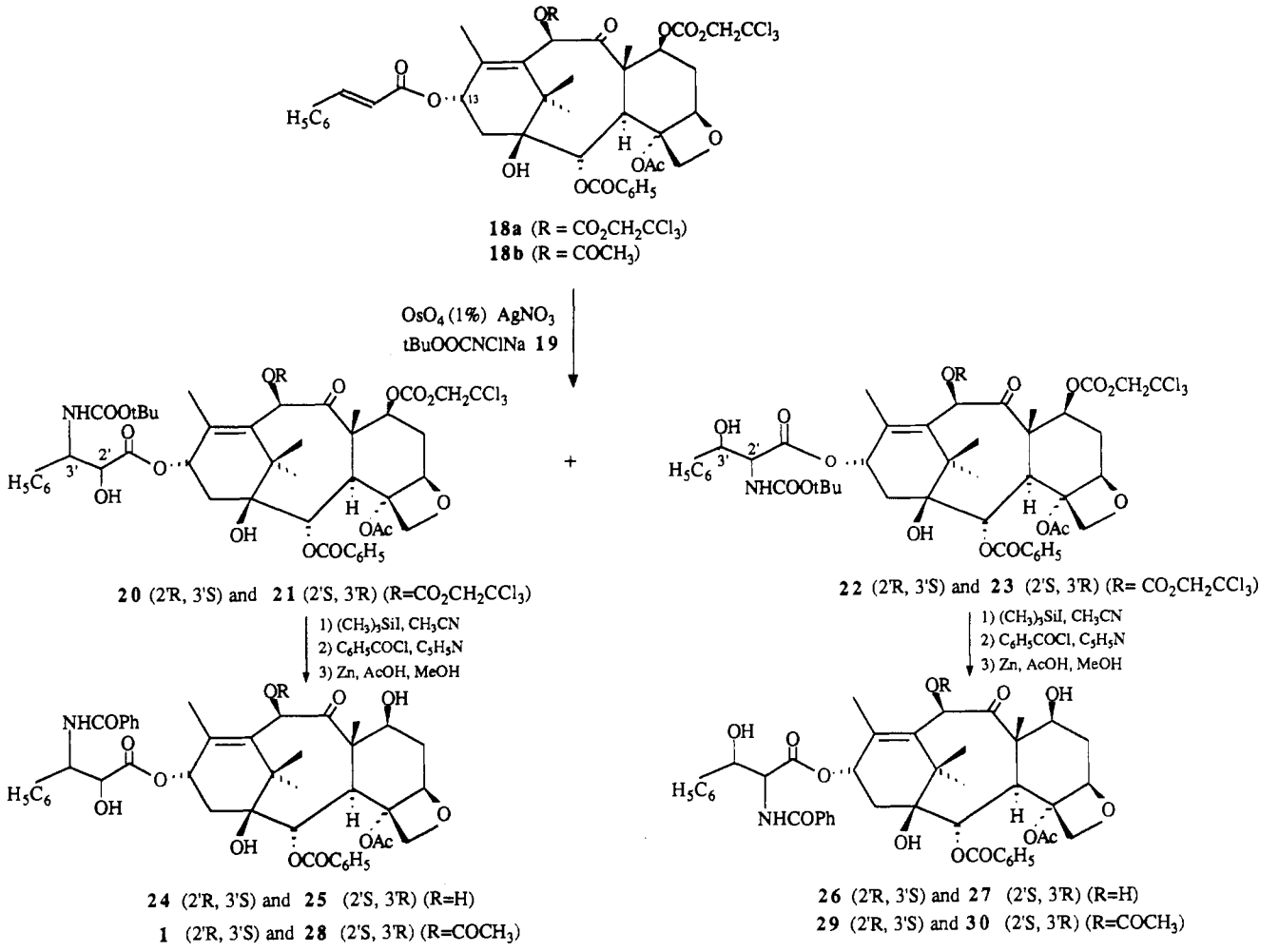
(29) Mangatal, L. Ph.D. Dissertation, 1989, Université Paris XI, Orsay.

(30) Swindell, C. S.; Krauss, N. E.; Horwitz, S. B.; Ringel, I. *J. Med. Chem.* **1991**, *34*, 1176–1184.

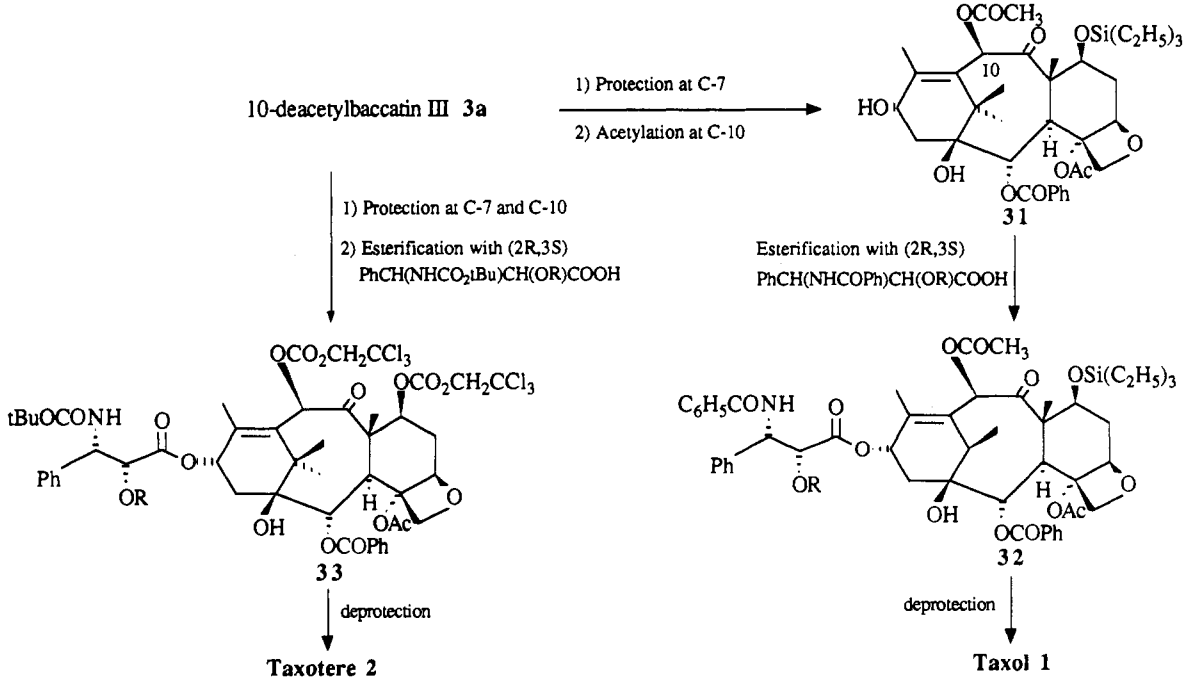
(31) Guéritte-Voegelein, F.; Mangatal, L.; Guénard, D.; Potier, P.; Guilhem, J.; Cesario, M.; Pascard, C. *Acta Crystallogr.* **1990**, *C46*, 781–784.

(32) (a) Falzone, C. J.; Benesi, A. J.; Lecomte, T. J. *Tetrahedron Lett.* **1992**, *33*, 1169–1172. (b) Chmurny, G. N.; Hilton, B. D.; Brobst, S.; Look, S. A.; Witherup, K. M.; Beutler, J. A. *J. Nat. Prod.* **1992**, *55*, 414–423. (c) Hilton, B. D.; Chmurny, G. N.; Muschik, G. M. *J. Nat. Prod.* **1992**, *55*, 1157–1161. (d) Baker, J. K. *Spectrosc. Lett.* **1992**, *25*, 31–48.

Scheme III. Partial Synthesis of Taxol (Oxyamination Procedure)



Scheme IV. Partial Synthesis of Taxol and Taxotere (Esterification with the Acid Side Chain)



Tubulins are a group of ubiquitous proteins responsible for the formation (inter alia) of the mitotic spindle during cell division. These proteins are in equilibrium with their assembled form: the microtubules. As previously noted, various drugs such as the "vinca" alkaloids and taxol act on the assembly–disassembly process leading to, respectively, disassembly of microtubules into tubulin and assembly of tubulin into

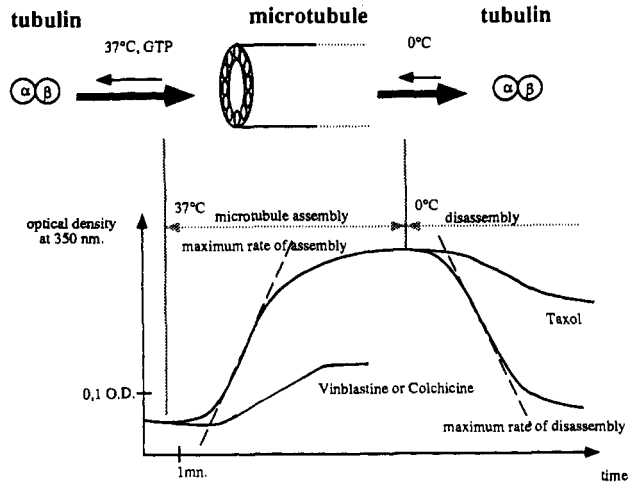


Figure 1. The tubulin assay.

microtubules. These "spindle poisons", as they are also named, are thus antitumor agents because they impair normal cell division.

Microtubule proteins are purified from mammalian brain by cycles of temperature-dependent assembly–diassembly,[33] and this equilibrium is monitored by using simple ultraviolet measurements at 350 nm (Figure 1). The maximum rate of disassembly is checked for each taxol analogue at various concentrations. The concentration of drugs inducing a 50% inhibition of the rate of diassembly (IC_{50}) at 0 °C is directly compared to the IC_{50} of taxol measured on the same day, under the same conditions. Practically, the IC_{50} values found for the taxol analogues ranged between 0.5- and 1000-fold that of taxol. This large "window" of values is not obtained in alternative assays and has allowed us to conduct efficient analysis of structure–activity relationships.

For taxol analogues, the "tubulin assay" seems to be more sensitive than measurements such as cytotoxicity, especially for compounds binding weakly to tubulin. The importance of this latter point is underlined by the fact that 10-deacetylbaccatin III **3a**, which is practically devoid of cytotoxicity, could be selected on the basis of its slight inhibition of the microtubule disassembly process. Furthermore, measurement of the activity on tubulin, directly connected to the affinity of the drug for tubulin, provides a powerful method to study in vitro structure–activity relationships.

Preliminary Studies. Preliminary structure–activity relationship studies have led to the conclusion that, whereas structural modifications at C-10 and/or C-7 have little effect on the activity of taxanes, the presence of a taxol-type side chain at position 13 is essential.[34] Though some variability is allowed within the side chain itself, a free hydroxyl group at the 2′-position is important (especially in vitro);[34c] active compounds with an ester group at C-2′ can be considered as "prodrugs". The importance of the oxetane ring in securing a correct binding of these derivatives on tubulin has been proposed.[35] It should also be noted that, using the activity-guided purification, no molecule was selected lacking the benzoate group at C-2 and/or the oxetane ring, in spite of the presence of such derivatives in the plant crude extract.

Determination of the "Active" Conformation. In baccatin III analogues, the taxane skeleton has a rigid conformation. In contrast, the side chain at C-13 has a high degree of freedom. This substructure, alone, has been analyzed by X-ray with various substituents at C-2′ and/or C-3′,[1,36,37] and it showed the same series of hydrogen bonding ($C_{1'}$ = O···2′-OH and 2′-OH···NH) as those seen in the side chain of Taxotere[24,30] (Figure 2).

Analysis of the crystal structure of Taxotere, crystallized from methanol, showed the presence of two distinct areas whose spatial arrangements occur as a result of interaction with neighboring molecules in the crystal. First, the polar functions at C-7, C-9, C-10, and, to a lesser extent, the oxetane ring constitute a hydrophilic area which is in contact with polar residues of neighboring Taxotere molecules. Secondly, the hydrophobic benzoate group at C-2 holds the side chain in a position where the *t*-BOC group is close to it (due to dispersion forces) and, consequently, reduces interactions with polar solvent molecules.

The X-ray analysis of Taxotere gave us information about the requisite conformation of a biologically active taxoid possessing a side chain at C-13. Although the stacking of the molecules in the crystal could be similar to those found in the interaction of a drug with its receptor, we endeavored to confirm this through NMR experiments, in various solvents ($CHCl_3$, CH_3OH) at different temperatures (−20, 20, 40 °C). Qualitative and quantitative experiments (nuclear Overhauser effect experiments: ROESY) on Taxotere and various structural analogues have been performed in order to compare the interhydrogen distances in the solid state and those obtained by NMR calculation or molecular modeling.[38] The results obtained show that there is a good correlation between the various methods used (X-ray, NMR, and molecular modeling). Thus, the distances obtained from NMR between the protons H2′, H3′ and C-18H_3, CH_3COO-C-4 are similar to those found using molecular modeling.

Thus, molecular modeling (using Macro Model) was used with confidence to predict the most suitable structure for each compound when X-ray data was not available. After minimization of the starting conformation of the studied compound, an automatic generation of conformers was performed (using a Monte Carlo method and MM2 as force field) on the free rotating bonds of the molecule, essentially the side chain at C-13, the benzoate at C-2, and the acetate at C-4. This experiment was achieved both in vacuo and in the presence of water molecules. The most stable conformers were analyzed for their interhydrogen distances with respect to NMR results and were compared with the X-ray structure of Taxotere. In most instances,

(33) Shelanski, M. L.; Gaskin, F.; Cantor, C. R. *Proc. Natl. Acad. Sci. U.S.A.* **1973**, *70*, 765–769.
(34) (a) Parness, J.; Kingston, D. G. I.; Powell, R. G.; Harracksing, C.; Horwitz, S. B. *Biochem. Biophys. Res. Commun.* **1982**, *105*, 1082–1089. (b) Lataste, H.; Senilh, V.; Wright, M.; Guénard, D.; Potier, P. *Proc. Natl. Acad. Sci. U.S.A.* **1984**, *81*, 4090–4094. (c) Mellado, W.; Magri, N. F.; Kingston, D. G. I.; Garcia-Arenas, R.; Orr, G. A.; Horwitz, S. B. *Biochem. Biophys. Res. Commun.* **1984**, *124*, 329–336.
(35) Kingston, D. G. I.; Magri, N. F.; Jitrangsri, C. *New Trends Nat. Prod. Chem.* **1986**, *26*, 219–234.
(36) Powell, R. G.; Miller, R. W.; Smith, C. R., Jr. *J. Chem. Soc., Chem. Commun.* **1979**, 102–104.
(37) Peterson, J. R. Do, H. D.; Rogers, R. D. *Pharm. Res.* **1991**, *8*, 908–912.
(38) Dubois, J; Guénard, D.; Guéritte-Voegelein, F.; Guedira, N.; Potier, P.; Beloeil, J. C.; Gillet, B. To be published.

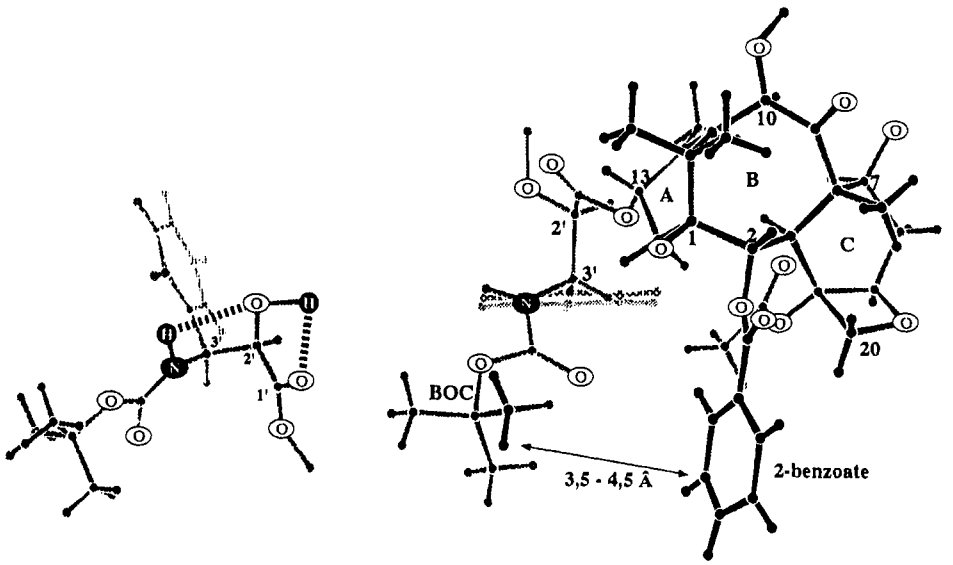
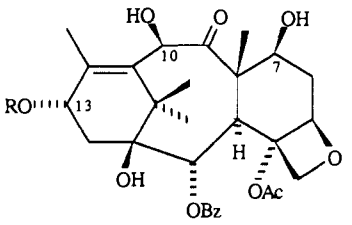


Figure 2. X-ray structure of Taxotere and C-13 taxotere side chain showing the hydrogen bonds.

Table I. Activity on Tubulin of Taxotere Analogues with Modified Side Chains



	R =	IC_{50}/IC_{50}(taxotere)*
2	Ph—CH(BOCNH)—CH(OH)—C(=O)—	1
34	Ph—CH(BOCNH)—CH(H)—C(=O)—	4,1
35	Ph—CH(H)—CH(OH)—C(=O)—	4,5
36	Ph—CH(H)—CH(H)—C(=O)—	17
37	H—CH(H)—CH(H)—C(=O)—	41

* The ratio IC_{50}/IC_{50} (Taxotere) gives the activity with regard to taxotere. IC_{50} is the concentration of the compounds leading to a 50% inhibition of the rate of microtubules disassembly.

there was a good correlation between the conformer of lowest energy, the NMR results, and the X-ray structure, showing that all active taxol analogues possess, in solution, a Taxotere-like conformation.

As the conformation of the side chain represents a major element for an efficient binding to tubulin, each functionality at C-2′ or C-3′ can act either by their influence on the molecular properties (i.e., conformation, hydrophilicity, dielectric constant, and so on) or by a direct interaction between these groups and the protein. The results obtained with the side chain analogues **34–37** provide an answer. First, the conformation of each compound is very similar to that of Taxotere **2**, and second, the increase in the activity in going from compound **37** to **2** is the product of separate contributions of the substituent at 2′ and 3′ (Table I). This observation demonstrates that successive additions of positive interaction between drug and tubulin stabilize their binding. It follows that the best "active" conformation of the side chain is represented by that of Taxotere as defined above.

The comparison between NMR and molecular modeling studies on the three stereoisomers of Taxotere (2′*R*,3′*R*; 2′*S*,3′*S*; 2′*S*,3′*R*) leads to the same conclusion: the biological activity increases when the conformation reaches that of Taxotere. In the case of taxol itself, the benzoyl group at C-3′ lies close to the benzoate at C-2, in a fashion similar to that of the *tert*-butyl group of Taxotere. As we showed earlier,[24] the unnatural 10-deacetylisotaxol (2′*S*,*R*) **25** is 4-fold less active than taxol in the tubulin disassembly assay. Molecular modeling shows that the structure of 10-deacetylisotaxol **25** is superimposable with the "active" conformation except that the 2′-OH group is in an opposite direction with no hydrogen bonding such as in the 2′-deoxy derivative **34**. In contrast, the conformation of iso-Taxotere (2′*S*,3′*R*), obtained from compound **21** (Scheme III) after removal of the protective groups, is quite different and can explain the weak activity of this compound.[24]

In the binding process to tubulin, it can be assumed that two structural features of taxol-like compounds are recognized. The hydrophobic area, including the benzoate group at C-2, will locate in the core of the protein. The phenyl group at 3′ and the hydroxyl group at 2′ could stabilize the drug–protein complex through a direct interaction between peptidic residues such as aromatic amino acids and the phenyl group and between polar functions of the protein and the 2′-hydroxyl group (Figure 3). This hypothesis is supported by the fact that the ester derivatives at C-2′ which possess a conformation similar to that of Taxotere have, however,

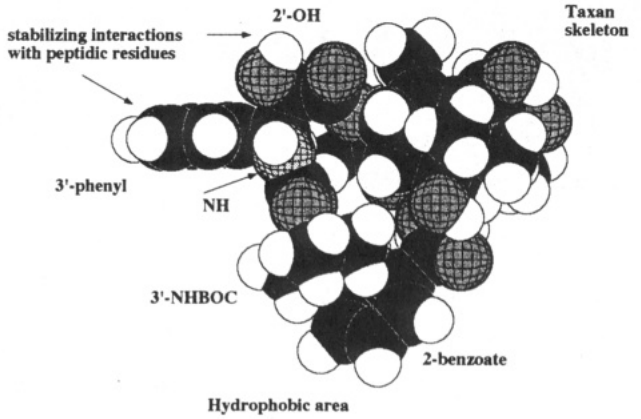


Figure 3. 3-D view of taxotere after minimization with MM2 (MacroModel) (representation by Moldraw, J. M. Cense).

a very weak affinity for tubulin.[34b,c] Consequently, the loss of "activity" of the C-2′ acetyl derivative is most probably due to the loss of a direct interaction of the 2′-OH group with a peptidic residue (on tubulin). Moreover, the polar area of the taxane ring (oxygenated functions at 7, 9, and 10) could be located outside the binding site close to the aqueous medium.

NMR and molecular modeling experiments confirm the nature of the hydrogen bond between the hydroxyl group at 2′ and the keto group at C-1′, defining the conformation of the side chain and the proximity of hydrophobic groups for the side chain location versus taxane skeleton. So, we can assume that the direct interaction of the side chain substituents with tubulin stabilizes or destabilizes the binding of the compound with tubulin.

It is important to know which structural elements of the molecule are responsible for the affinity with tubulin. Various chemical groups could be added to the hydroxyl groups at C-7 or C-10, but a covalent marker at this place would obviously be of poor utility for binding site recognition studies. The importance of 2′-OH and 3′-phenyl have already been emphasized, and concerning the nature of the amide substitution at 3′, many hydrophobic substituents could be proposed; this group will locate under the "umbrella" of the 2-benzoate. Bulky functionalities, such as fluorescent probes, can be added at this position without loss of affinity for the taxol binding site.

The study of the metabolites of taxol in rat and human has shown that the structural modifications are mainly located on the 3′-phenyl group in the para position and then on the meta position of the benzoate at C-2, showing the accessibility of these groups.[39,40]

Concluding Remarks

The emergence of taxol as one of the most promising drugs in cancer chemotherapy and the problem of its limited supply have led to an increase in the chemical and biological studies of the "taxoids". A major result of our work, besides the first successful partial synthesis of taxol, was the discovery of a new antimitotic compound: Taxotere, which has shown better bioavailability[25b] than taxol and superior activity over taxol in some in vitro[41] and in vivo[26] assays. Taxotere (as well as taxol) can be produced in good yield from a natural precursor, 10-deacetylbaccatin III, obtained from the leaves of a number of species of *Taxus*. From that substance, various derivatives have been prepared, allowing the study of structure–activity relationships in the "taxoid" series. Among all the compounds studied, Taxotere possesses the best affinity for its cellular receptor, tubulin. Besides its clinical and chemical interest, taxol is a very efficient tool for biological and cellular studies. Indeed, because of its specific binding to tubulin, inducing the assembly process, taxol and its analogues could be considered as possible exogene models of the cytoplasmic constituents implicated in the regulation of the tubulin–microtubule equilibrium, which is a major process of cell division.

We are deeply grateful to our colleagues who have made important contributions to this work: Michel Colin, Joëlle Dubois, Augustin Hittinger, Marie-Thérèse Le Goff, Lydie Mangatal, Françoise Picot, Veronique Senilh-Gastiger, and Anne Walh. The authors wish to thank Jean-Claude Beloeil and Brigitte Gillet (CNRS, ICSN, Gif-sur-Yvette), Michel Wright and Bernard Monsarrat (CNRS, LPTF, Toulouse), Andrew Greene and Jean-Noel Denis (CNRS, LEDSS, Grenoble), François Lavelle and Marie-Christine Bissery (Rhône-Poulenc Rorer, Vitry), and their co-workers for their collaboration. The authors thank Dr. Aloysius Siriwardena for his help in the preparation of this manuscript and acknowledge support from Rhône-Poulenc Rorer and the Association pour la Recherche sur le Cancer and the Ligue Nationale contre le Cancer.

(39) Monsarrat, B.; Mariel, E.; Cros, S.; Garres, M.; Guenard, D.; Gueritte-Voegelein, F.; Wright, M. *Drug Metab. Dispos.* **1990**, *18*, 895–901.

(40) Montsarrat, B.; Alvinerie, P.; Garès, M.; Wright, M.; Dubois, D.; Guéritte-Voegelein, F.; Guénard, D.; Donehower, R.; Rowinski, E. *J. Cell. Pharmacol.*, in press.

(41) Aapro, M.; Braakhuis, B.; Dietel, M.; Hanauske, A.; Hill, B.; Kelland, L.; Lelievand, P.; Silvestrini, R.; Zoli, W. *Proc. Am. Assoc. Cancer Res.* **1992**, *33*, 3086.