RHÔNE-POULENC LECTURE*
Search and Discovery of New Antitumour Compounds

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1 Introduction

Until the latter part of the nineteenth century, plants, minerals, and, more rarely, animals constituted the major sources of drugs. Consequently, natural products form the basis of therapeutics. For more than a century the pharmaceutical industry maintained an important effort in trying to select new and interesting drugs from Nature but since 1970 this field of research has been practically abandoned. However, the tremendous progress realized in biology and pharmacology in recent times advocates another look at 'old drugs'. Although some of these have been known for a very long time, knowledge of their pharmacological profile is often limited and would certainly be increased by looking at them from a new perspective. Furthermore, most of the natural products so far structurally characterized (and sometimes synthesized) would have never been created, even by the most ingenious chemist. The structure of morphine, for instance, is not that complex; however, we can hardly imagine this structure ever being synthesized *de novo* by Man.

There are other facts that should encourage us to continue to study the natural world:

Fewer than 10% of the plants on our planet have been examined for their biological properties. This '*terra incognita*' is therefore immense.

Among the major drugs which have been discovered in recent times, several have been isolated from natural sources: immunomodulating substances (cyclosporin, FK 506), inhibitors of the biosynthesis of cholesterol, new antibiotics, new antitumour compounds, compounds interacting with the nervous system, *etc.*

2 Search and Discovery of New Antitumour Compounds

Cancers and related diseases represent the second major cause of death for Man. The aetiology of cancers is multiple but great progress has been made in recent times towards the understanding of these diseases with the discovery of oncogenes. The links between these genetic 'templates', their expression products, and the various growth or differentiation factors are becoming more and more important. Such discoveries will undoubtedly lead to more rational approaches to the treatment of cancerous diseases.

Pierre Potier was born near Paris in 1934 and obtained his Diploma in Pharmacy (1957) and Ph.D. (1960) from the University of Paris. He has been, since 1962, at the Institut de Chimie des Substances Naturelles at Gif-sur-Yvette with Professors M.-M. Janot, E. Lederer, D. H. R. Barton, and G. Ourisson. He became Director of this Institute in 1990 as well as, in the same year, Professor of Chemistry at the Muséum National d'Histoire Naturelle in Paris. He has been a Visiting Professor at the University of Strathclyde (1984—1990). Professor Potier has published more than 300 papers in the fields of the chemistry of natural products, organic chemistry, and medicinal chemistry. He is a member of the French Academy of Sciences.

For the time being, surgery, radiotherapy, and chemotherapy remain the three most important methods of treating cancers. Immunotherapy is still in its infancy but remains a serious hope. Cancer chemotherapy relies principally on the use of a number of natural products, intact or chemically modified, and secondly, on purely synthetic products (some of them, methotrexate for instance, being the mimics of naturally occurring substances). These compounds have been classified according to their known (or supposed) mode of action:

(i) *the alkylating agents* which are given to 'alkylate' several crucial biological targets (nucleic acids, for example) impairing their normal mode of functioning. These agents are: cyclophosphamide, nitrogen mustards, platinum derivatives, *etc.*
(ii) *the intercalating agents* whose activity is based upon their 'intercalation' between the base pairs of nucleic acids (mostly, but certainly not exclusively, DNA). Examples of such compounds are adriamycin, platinum derivatives, and bleomycin.
(iii) *the antimetabolites* which are compounds that play the role of lures *vis-à-vis* metabolic processes: examples are methotrexate, fluoro-uracil *etc.*
(iv) *the spindle poisons* which either prevent the formation of the spindle (colchicine, maytansine, vinblastine-type compounds) or stabilize it (taxol and derivatives) during the cell division.

We have chosen to work on the intercalating agents and, more intensively, on the spindle poisons.

3 Antitumour Compounds of the Ellipticine Group

Ellipticine (1) is an alkaloid isolated in 1959 by Horning, Goodwin, and co-workers[1] from the leaves of *Ochrosia elliptica Labill*, (Apocynaceae) and found, since then, with its congener, olivacine (4) in many other plant sources belonging to the Apocynaceae and, less frequently, to the Loganiaceae families. *Ochrosia oppositifolia* (Lmk) K. Schum now known as *Neisosperma oppositifolia* (Lmk) Forsburg, Sachet, Boiteau was described by Rumphius (von Rumpf) in 1742 who reported its use in folk medicine against cancers of the nose.[2] A review of this subject has been published.[3]

Ellipticine (1) and 9-methoxyellipticine (2) have been found to be highly active compounds on various experimental tumours and leukaemias; 9-methoxyellipticine is, at the moment, one of the substances having the broadest spectrum of antitumour activity. Our first chemical studies of this series of compounds were made by Poisson, Le Men, and Dat-Xuong in collaboration with biochemists, biologists, and physicians (Paoletti, Le Pecq, Mathé, and their co-workers). They were quickly able to demonstrate that DNAs were the biological target of this type of molecule. They focused their efforts on Céliptium® (3) which they found to be the most active compound of the series. This compound has been marketed but has had a rather limited career in cancer chemotherapy. This outcome does not necessarily mean that compounds of the ellipticine series [or of the

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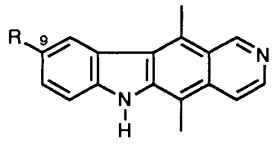
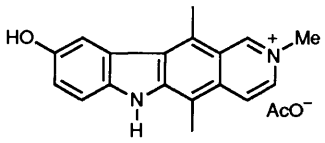
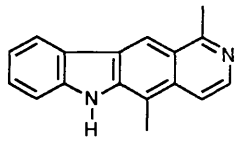


(1) R = H Ellipticine
(2) R = OMe



(3) Cêliptium®



(4) Olivacine

olivacine (4) series] cannot find their place in the armamentarium of cancer chemotherapy, and other compounds of this series should be evaluated. The mechanism of action of ellipticine *in vivo* has been a matter of considerable speculation more or less supported by experimentation, but which has been rarely confirmed. Ellipticine derivatives generally bind to double-stranded DNA (10^{-5} to 10^{-6} M), destroying kinoplastic DNA and base-pairing ability, thus denaturing DNA (for a review, see reference 3). The high affinity of ellipticine and its derivatives for DNA and their intercalation between DNA base pairs have long been considered the two main factors responsible for their antitumour activity. However, certain derivatives such as the 9-amino or 9-fluoroellipticine, although exhibiting quite a high affinity for DNA, have little or no *in vivo* activity. The strong activity of 9-hydroxyellipticine (2), an easily accessible metabolite of ellipticine, suggests that ellipticine (1) has to be metabolized to this compound before being active. This metabolization is enhanced in patients receiving barbiturates, which are known to be potent cytochrome P-450 inducers. Taking into account that ellipticine has to be oxidized *in vivo* to be active, Dat-Xuong prepared Cêliptium® (3), a compound having a high affinity for DNA preparations.

However, a hypothesis has been put forward that over-oxidation of 9-hydroxyellipticine or Cêliptium® occurs to give an imino-quinone system (see Figure 1). This reacts readily with various nucleophiles such as pyridine, amino acids, α-substituted primary amines, sulfydryl derivatives, methanol, ethyleneglycol, ribonucleosides, and ribonucleotides to give the corresponding and rather stable adducts.

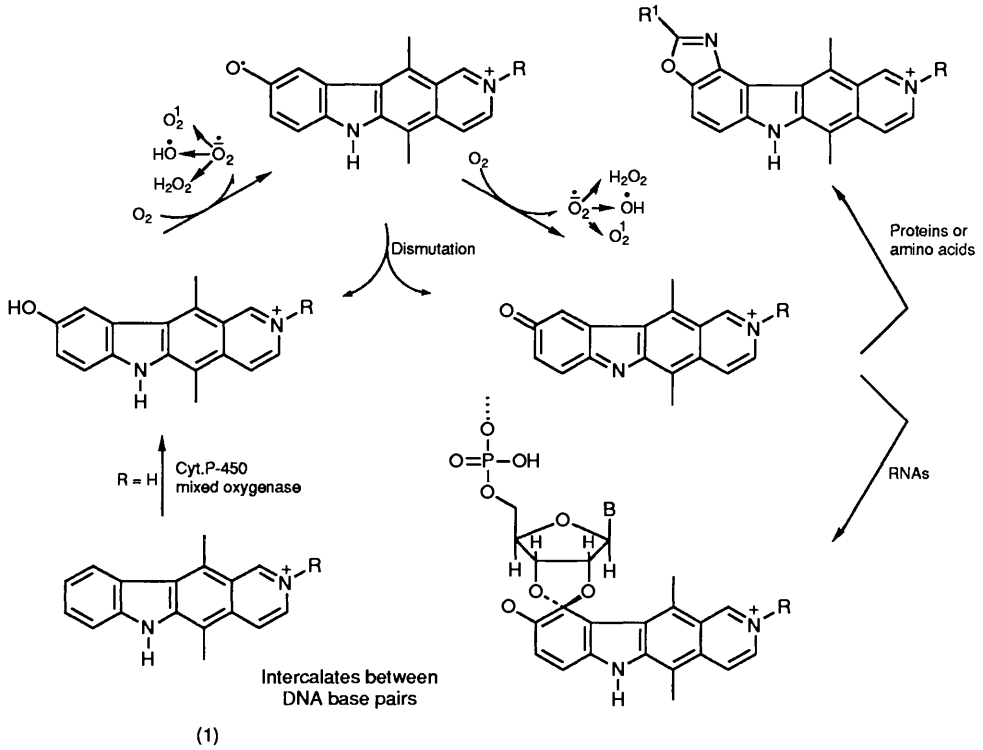
It is important to emphasize that some of these reactions (or their equivalent) can take place *in vivo* and thus explain at least a part of the biological activity of ellipticine and structurally related compounds [in particular all those able to lead to an iminoquinone (or quinoid) structure].

A given antitumour agent has to penetrate the cell, cross the cytoplasm, and enter the nucleus before being able to interfere with the DNA structures in the nucleus (unprotected by their usual chromatin). Upon crossing the cytoplasm they can well encounter other nucleic acid structures such as the RNAs, whose role is of crucial importance in the transfer of genetic information, protein synthesis, and a number of other basic biological processes (co-factors, ATP, GTP, *etc.*). The high reactivity of ribonucleosides or ribonucleotides with 9-hydroxyellipticine derivatives under oxidative conditions leading to the formation of regiospecific and stereoselective ketal linkages between 2′ and 3′ hydroxyl groups of the ribose moiety of these compounds and the 10-position of ellipticine derivatives, suggests the occurrence of these reactions *in vivo*. Among the four naturally occurring ribonucleosides used in this reaction [A, G, T(or U), C], the order of reactivity for hydroxyellipticine is: A > G > > > T(U) > C. The decreasing reactivity in going from purines to pyrimidines is probably due to a better affinity ('recognition') of adenine and guanine for the pyrido-carbazole moiety of ellipticine, as has been shown by NMR measurements.[3] The poly-A tail of the m-RNAs, or the 'cap' present at the 5′ end of m-RNAs, are obvious targets for such reactions.

The formation of dioxolanes (with ribose or related glycolic structures) and oxazolines (with amino acids) are examples of the formation of *reversible covalent bonds* which play an important role in the control of many biological processes. These bonds can easily be broken by slight changes of pH (or redox conditions), which can occur on passing from one cellular compartment to another.

There are many biomolecules which can participate in such

Figure 1



(1)

reaction processes, in particular those able to generate iminoquinoid systems (or their equivalent) by simple oxidation processes (serotonin, catecholamines, some steroid hormones, *etc.*). It is of interest to determine whether these highly bioactive substances can interact with proteins, nucleotide structures (or both in a '*ménage à trois*'), thereby temporarily blocking their function (or expression).

4 New Spindle Poisons

One of the essential features of cell division is the formation (and disappearance) of the mitotic (or meiotic) spindle. The spindle plays an important role in the distribution of the chromosomes to the two daughter-cells resulting from normal cell division. Some natural products are known to impair cell division. Colchicine (5) was the first of these to be discovered; its pharmacological action is that of the plant which contains it, *Colchicum autumnale* L. (Liliaceae), used for the treatment of gout. Its biological target has been relatively recently identified as tubulin, a ubiquitous protein present in all eukaryotic organisms. Tubulin is a heterodimeric protein (2 × 55 000 daltons) which polymerizes (GTPase activity) leading to microtubules and microfilaments which, after further assembly, constitute the spindle.[4] Colchicine prevents the polymerization of tubulin into microtubules. Other natural products are also known to interfere with the formation of the spindle: podophyllotoxin (6), vinblastine (7) [or vincristine (8)], taxol (9), and their derivatives, *etc.*

Microtubules are generally accompanied by the so called 'MAPs' (*M*icrotubule *A*ssociated *P*roteins). These are proteins which play an important role in the various biological functions of microtubules or microfilaments (*i.e.* axonal transport, brain organization, hormone secretion, motility of various cells, *etc.*). One of them, τ (tau) protein, has been recently shown to be involved in some degenerative nervous disorders such as Alzheimer's disease. These proteins may also have other important functions.[5]

Tubulin constitutes the major, if not unique, target of the spindle poisons, which inhibit polymerization of tubulin into microtubules or, less frequently, its depolymerization [like taxol(9)]. This property forms the basis of a simple and rapid biological test which can be used for selecting new spindle poisons. The test consists of measuring inhibition of polymerization (or depolymerization) of a preparation of tubulin (generally extracted from pig or sheep brain) by increasing quantities of a potential inhibitor.

We have developed this test, based on Shelanski's method,[6] and have found that it considerably shortens the time required to perform classical pharmacological testing on *in vitro* and *in vivo* preparations. It spares the lives of many animals as evaluation on animal tumours can then be restricted to those products being found active in the 'tubulin test'.

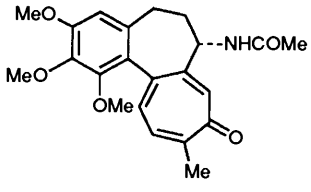
4.1 Navelbine®

We first applied this screening method to the antitumour alkaloids of the vinblastine group.[7] The biomimetic-type synthesis of this group of structurally complex natural products was achieved for the first time in our laboratory as early as 1974.[8] This synthesis is based on the modified Polonovski reaction[9] which uses the trifluoroacetic anhydride in place of the acetic anhydride originally used in the genuine Polonovski reaction. The imminium ion which is formed in the first stage of the reaction is stable in the presence of trifluoroacetate ion while, in the Polonovski reaction, this imminium suffers from the attack of the acetate ions. The 'dimeric' alkaloids of the vinblastine group (7) are formed by the coupling of two structural subunits, catharanthine (10) and vindoline (11), which are present in the same plant, the 'Madagascan periwinkle' [*Catharanthus roseus* G. Don (Apocynaceae)] and related species.[7]

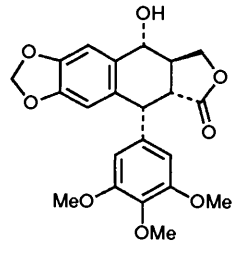
When catharanthine *N*-oxide (12) is treated with trifluoroacetic anhydride in the presence of vindoline (11), one gets, after reduction *in situ*, anhydrovinblastine (13) as a major product, possessing the 16′*S* configuration of the natural products. All previous synthetic efforts led invariably to the biologically inactive 16′*R* configuration (Scheme 1). Anhydrovinblastine (13) constitutes the precursor of a number of other 'dimeric' indole alkaloids. Vinblastine (7) and vincristine (8) are obtained differently;[7] they are among the most often used compounds in the field of cancer chemotherapy. Our efforts were partly motivated by the prices of these drugs (3 M$/kg for vinblastine and 20 M$/kg for vincristine!). Also, synthesis has made available compounds otherwise inaccessible by direct structural modifications of the natural products *e.g.* Navelbine® or noranhydrovinblastine (14)[10] (Scheme 2).

Navelbine® is currently used in the treatment of non-small-cell lung cancer and of breast cancer. It is orally active and its use will almost certainly be extended to the treatment of other cancerous diseases.

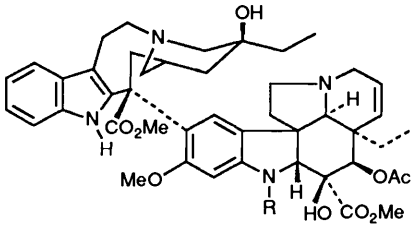
Vinblastine-type compounds interact, like other spindle poisons, with tubulin. The compounds have to be biosynthesized in such a way as to prevent them from reacting with the tubulin of



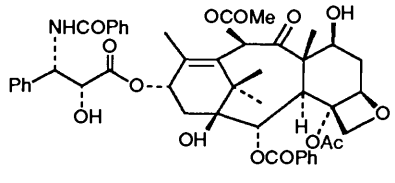
(5) Colchicine



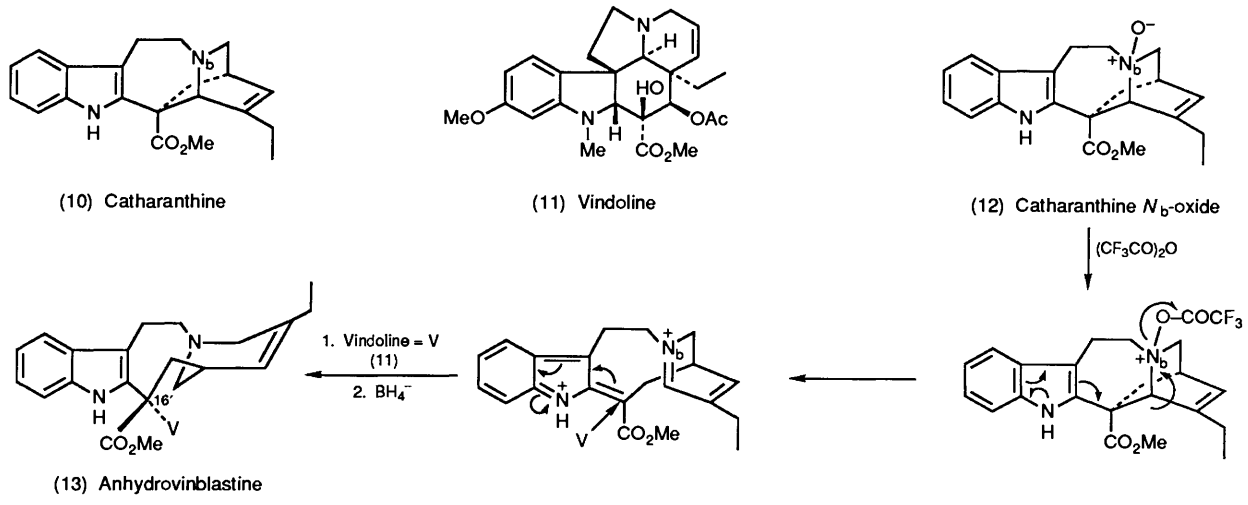
(6) Podophyllotoxine



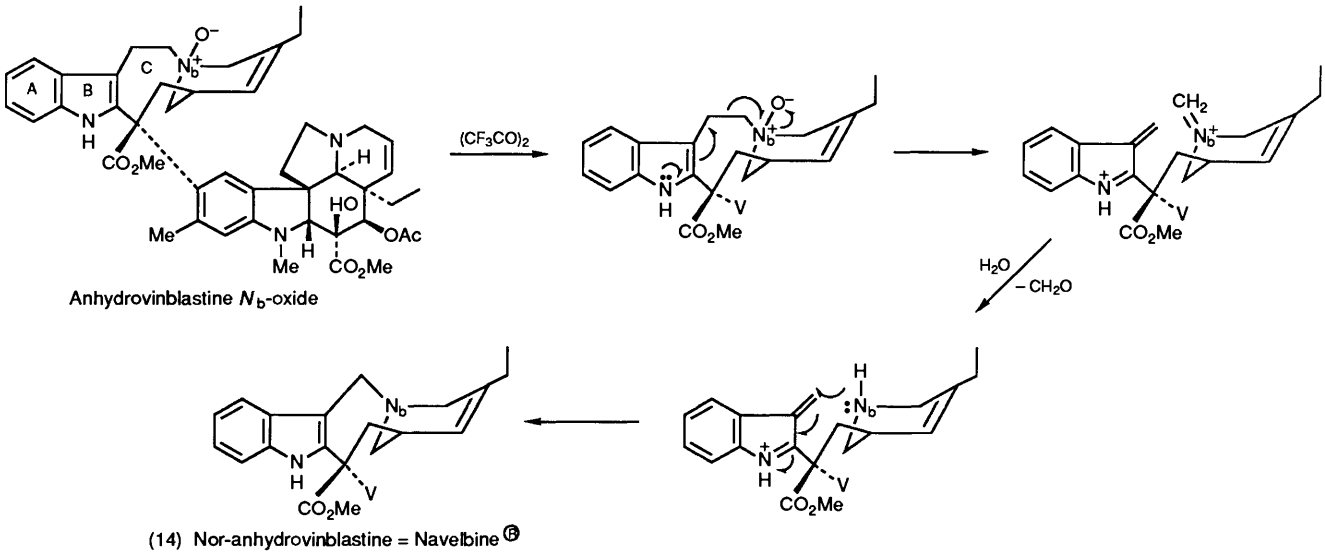
(7) (R = Me) Vinblastine
(8) (R = CHO) Vincristine



(9) Taxol



Scheme 1



Scheme 2

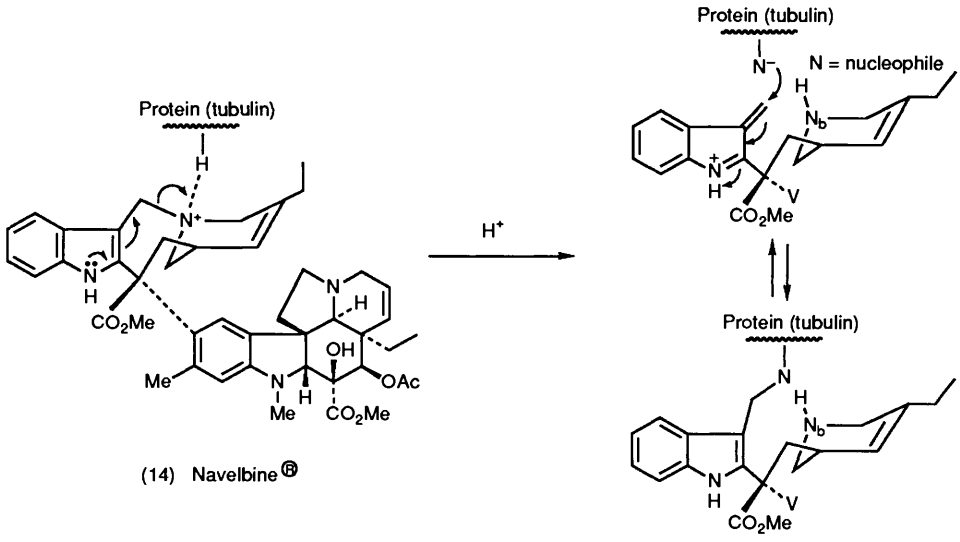
the plant which produces them. Otherwise, vinblastine would be toxic to *Catharanthus spp.*, colchicine to *Colchicum, etc.* just as animal venoms should also be toxic to the animals which produce them! This problem is avoided by vinblastine and related compounds being biosynthesized and localized in vacuoles. There is no definitive proof that cytochrome P450-type enzymes are responsible for the coupling reaction between catharanthine (10) and vindoline (11) leading, after reduction (NADPH ?) to vinblastine-type compounds, although these enzymatic systems are known to be present in (or around) the vacuoles.

Navelbine® (14) is formed from anhydrovinblastine (13), one of the biogenetic precursors of other dimeric alkaloids of *Catharanthus spp.* We think that Navelbine® (14) is probably not a naturally occurring compound: in order to obtain Navelbine,® anhydrovinblastine has to be transformed by a modified Polonovski reaction, implying the formation of the N_b'-oxide of anhydrovinblastine (or its biogenetic equivalent) followed by a fragmentation reaction (Scheme 2). However, the pH of the vacuoles is acidic,[11] as shown by ^{31}P NMR measurements, which suggests that N_b' of anhydrovinblastine is protonated and not prone to further reaction.

The biological mode of action of Navelbine® resembles that of other spindle poisons of the vinblastine group; however, it has structural peculiarities which can partly or entirely explain the distinct pharmacological profile of this new antitumour agent. Thus Navelbine® contains an *eight*-membered ring instead of the *nine*-membered ring found in vinblastine or related compounds. One knows[10] that N_b' of vinblastine-type compounds must be 'free' (protonated?) for these to exhibit antitumour activity. Whatever the exact mode of interaction of these types of compounds with tubulin, it is quite probable that after their 'recognition' by tubulin (hydrophobic interactions) further interactions come into play and protonation of N_b' of the upper part of vinblastine-type compounds by an amino acid of tubulin is possible. In the case of vinblastine-type compounds, there is no further consequence of this protonation. In the case of Navelbine®, however, protonation of N_b' of the 'gramine portion' of that alkaloid (instead of the 'tryptamine portion' in the equivalent vinblastine-type compounds) can be followed by a fragmentation reaction which offers the possibility of nucleophilic addition of a suitable group of tubulin onto the newly formed conjugated imminium indolic ion (see Scheme 3). This newly formed bond is a *reversible covalent bond* whose role should be important in the control of the fate of tubulin, microtubules, spindle, *etc.* (see above concerning the formation of ellipticine derivatives with ribonucleosides or nucleotides).

4.2 Taxotère®

Taxus baccata L. (Taxaceae) is the most commonly encountered species of yew tree in Europe. There are other species of *Taxus* (or related genus) all over the world, but they do not appear to differ significantly in their content of secondary metabolites. The



Scheme 3

wood of the yew tree is dense, imputrescible, and mould-resistant, and has been largely used for making bows [those of the battle of Crécy (1346) for instance] and furniture. However, it is slow growing and one or two centuries are necessary to get a good specimen of tree. Its toxicity has been known since Antiquity. Practically all parts of the tree are toxic with the exception of the red fleshy envelope of the fruit which is eaten by birds (and the seeds are therefore disseminated).

The first reported work on *Taxus* constituents is due to Lucas[12] who, in 1856, isolated from the leaves a toxic alkaloid named taxin.

In 1921 Winterstein[13,14] identified a degradation product of taxin – 3-dimethylamino-3-phenyl propionic acid (15) – known as 'Winterstein's acid'. Lythgoe and co-workers[15] showed that the skeleton of taxin called taxane, is of diterpenic nature. It was only in 1971 that Wall and co-workers[16] isolated taxol (16) from the stem bark of an American yew, *Taxus brevifolia* Nutt., and disclosed its rather complex chemical structure. Taxol was later isolated from various other *Taxus* species, although in relatively low yields (0.1—0.2 g/kg of stem bark). It possesses a cytotoxic activity due to its unique mode of action on the microtubule proteins responsible for the formation of the spindle during cell division. While all other known spindle poisons have been shown to interfere with the polymerization of tubulin (see above), taxol and its derivatives are known to stabilize the spindle or to promote the assembly of microtubules into microfilaments and, finally, into the spindle. For a review on this topic, see reference 17.

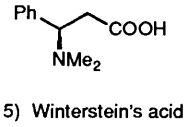
The cytotoxic properties of taxol were recognized by Wall and co-workers[16] on KB cells and, later, on leukaemias L1210, P388, and HeLa cells. This activity has been related to the interaction of taxol with the tubulin–microtubule system.[18] Taxol and derivatives are the only products known to favour microtubule assembly into microfilaments and spindle; they stabilize the spindle which, after cell division, should normally vanish. Taxol has been submitted for clinical evaluation (phases I and II) in both the United States and France and it appears to be an exceptionally promising drug. It has a very broad spectrum of activity against leukaemias and solid tumours and has already been successfully used in the treatment of ovarian cancers where other therapies proved to be ineffective.

Taxol is extracted from the stem bark of several species of yew (*Taxus* spp). However, the isolation procedure is tedious, low-yielding, and an obvious ecological problem. There is a widespread concern that 'if taxol proves effective . . . the yew population could be so severely depleted that there would not be enough trees left to make treatment successful'. Obviously, other sources have to be found to meet with the expected increasing demand. The National Cancer Institute of the USA has contracted for 27 000 kg of yew stem-bark.[19]

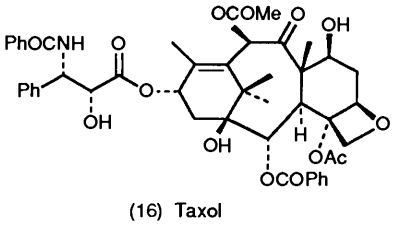
Attempts to achieve a total synthesis of taxol have not succeeded so far. However, we have found[19] an efficient solution to the problem of taxol supply. We analysed the various parts of *Taxus baccata* and isolated 10-deacetylbaccatin III (17), from the leaves of this species. These are quickly regenerated and can be harvested in rather large amounts without detrimental effects on the yew population. The production of taxol (16) (and derivatives) from 10-deacetylbaccatin III (17) is simple. Selective protection of (17) at the C-7 position (triethylsilylation) and acetylation at the C-10 position is followed by forced acylation of the secondary alcohol at C-13 (largely unreactive under normal conditions) by the suitably protected *N*-benzoyl phenylisoserine side chain. Deprotection of both the C-7 and C-2′ secondary alcohol functions leads to taxol (16)[19] (Scheme 4).

In another approach[20] 10-deacetylbaccatin III (17), suitably protected at both the C-7 and C-10 positions is converted into the cinnamoyl ester at the C-13 position. The cinnamic double bond is oxyminated (Sharpless method), leading to the four different possible isomers (Scheme 5). The 2′*R*,3′*S* isomer is then conveniently deprotected and *N*-benzoylated to give taxol (16). The poor diastereoselectivity of this method can be improved by using chiral ligands during the oxy-amination reaction.

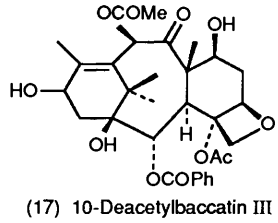
This approach led to diastereomers of taxol necessary for Structure–Activity Relationship studies. In addition to taxol (16), we were able to obtain several derivatives, one of which was given the name Taxotère® (18) and has revealed interesting



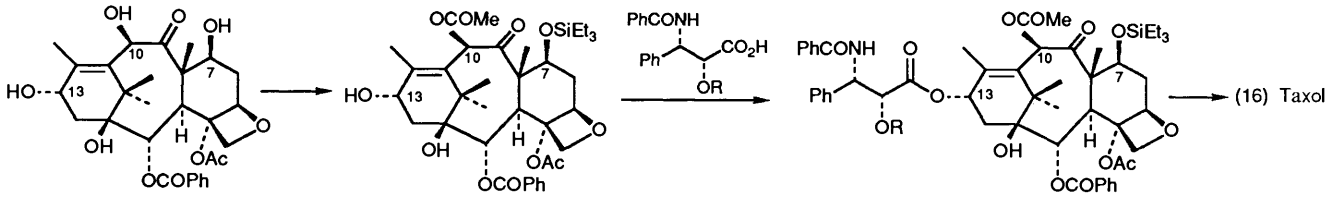
(15) Winterstein's acid



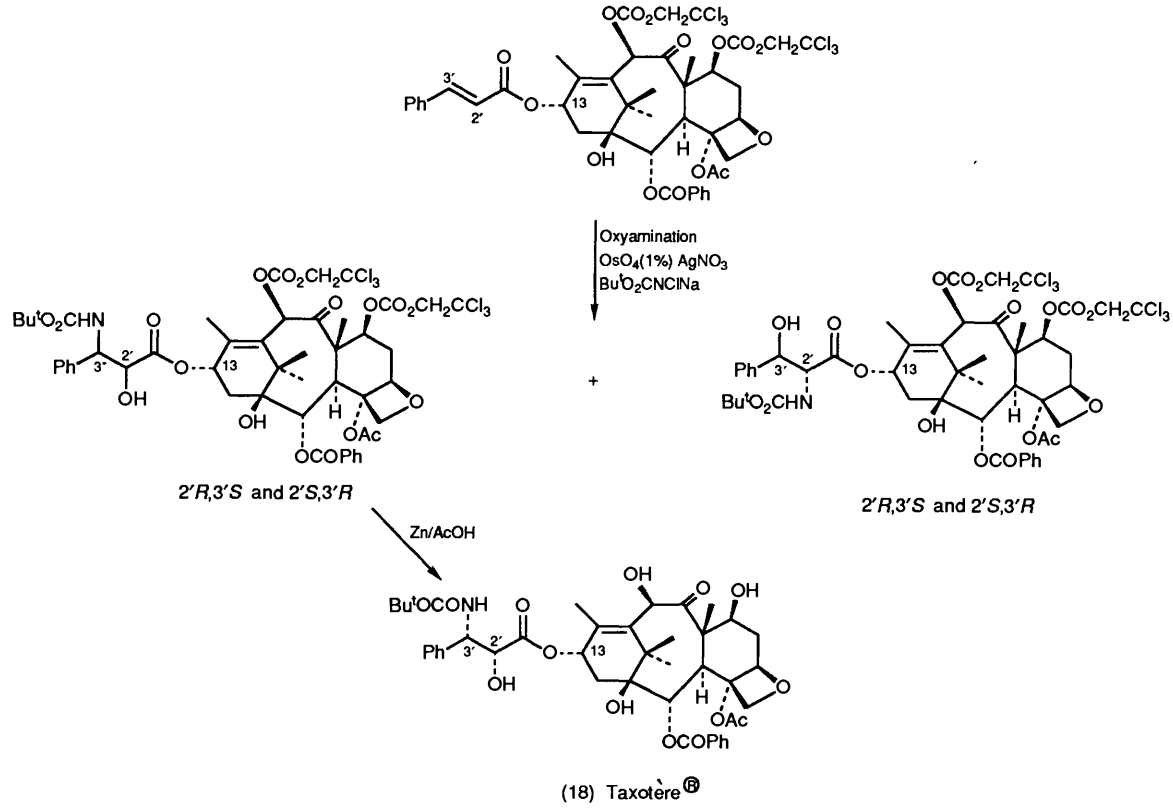
(16) Taxol



(17) 10-Deacetylbaccatin III



Scheme 4



Scheme 5

pharmacological properties.[21,22] It has better bioavailability and pharmacological characteristics than taxol and is a promising new anticancer agent (Scheme 6).

The exact mode of action of taxol derivatives is not clearly understood. Although tubulin constitutes its major (if not unique) biological target, it remains to be seen whether some of the Microtubule Associated Proteins (MAP's) play a role in the interaction between taxol derivatives and tubulin. Work is in progress which will allow a better understanding of the very promising therapeutic activity of this type of compound.[4]

5 Conclusion and Prospects

Arthur Kornberg has written:[23]

'Much of life can be understood in rational terms if expressed in the language of chemistry. It is an international language, a language for all time, and a language that explains where we came from, what we are, and where the physical world will allow us to go. Chemical language has great esthetic beauty and links the physical sciences to the biological sciences. Unfortunately, the full use of this language to understand life processes is hindered by a gulf that separates chemistry and biology. This gulf is not nearly as wide as the one between the humanities and sciences. Yet, chemistry and biology are two distinctive cultures and the rift between them is serious, generally unappreciated and counterproductive.'

In this article we have tried to show that medicinal chemistry constitutes an unlimited field of research open to those being chemists and biologists at the same time: the biochemists of modern times. There is no logical boundary between chemistry and biology and careful chemical analysis of biological processes will continue to lead to fundamental discoveries. One consequence of those discoveries will inevitably be progress in therapeutics.

6 References

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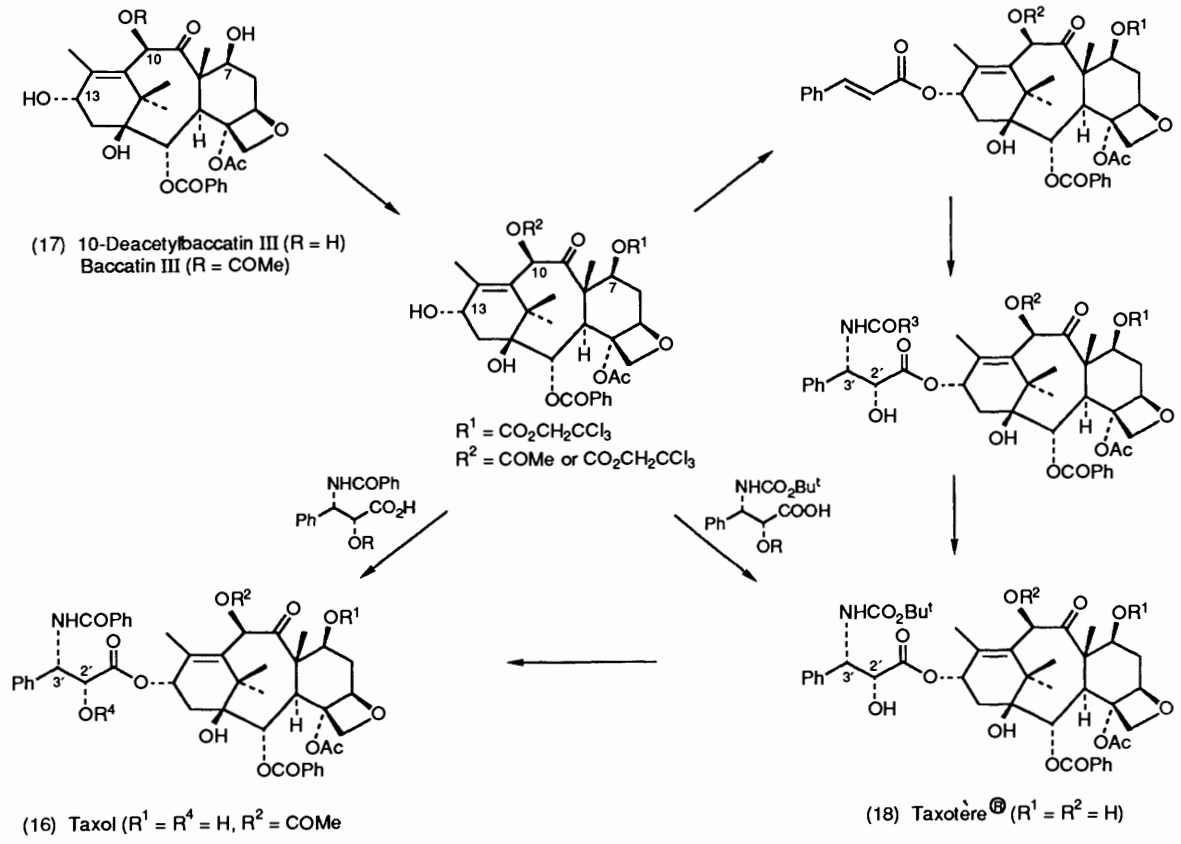

Scheme 6

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