International Organization for Chemical Sciences in Development

Working Group on Plant Chemistry

CHEMISTRY, BIOLOGICAL AND PHARMACOLOGICAL PROPERTIES OF AFRICAN MEDICINAL PLANTS

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4. The search for, and discovery of, two new antitumor drugs, Navelbine and Taxotere, modified natural products

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Natural products, a never out-dated subject

Man, since time immemorial, has always sought to extract subsistence and medicines from his environment. It is the accumulation of this experience acquired over several millennia in the selection of remedies, many of which have now been forgotten, which constitutes, even today, the basis of therapeutics. Names such as *Colchicum*, *Digitalis*, *Belladonna*, poppies and opium, *Cinchona*, strychnine, etc. are very current.

Natural products still represent today more than 80% of pharmacological and therapeutic lead compounds. This high percentage is the result not only of what I call archeopharmacology (that based on ancient remedies) but also of the discovery, every year, of several natural substances having major biological and therapeutic interest. These include new antibiotics, of course, antiparasitics, compounds acting on the nervous and cardiovascular systems, etc.

A remarkable advance was the discovery, accidental as is often the case, of cyclosporin, a widely-used drug for the prevention of transplanted organ rejection. This also allowed the development of screening tests for substances of this type and led to the discovery of other immunomodulating compounds such as FK 506, discovered in Japan, as well as rapamycin. Investigation of the mechanism of action of these substances has very recently revealed some of the mysteries of immunology.

Natural products thus constitute, today and for a long time to come, a practically endless source of novel substances able to enrich therapeutics. I would

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like to give you two examples chosen from the many research programs conducted over twenty years in our Institute at Gif and whose goal is the discovery of new substances of therapeutic interest.

The discovery of Navelbine®, an antitumor drug.

The first example is that of substances isolated from the Madagascan periwinkle, *Catharanthus roseus* G. Don (Apocynaceae). The first sample of this plant brought to France is conserved at the National Museum of Natural History in Paris. It was provided by Etienne de Flacourt (1607-1660), a colonizer sent by the Maréchal de la Meilleraye to pacify the south of Madagascar where the colonial residents of Fort-Dauphin, who worked for the East India Company, had become agitated. Monsieur de Flacourt made use of his mission of pacification to study this country and its nature. As a result, he wrote several books, including a "Dictionary of the Language of Madagascar". In fact, even at that time, numerous dialects existed in Madagascar and the language spoken at Fort-Dauphin (in the Southeast of the island) was not the same as that spoken on the high merina plateaus or in the north (where Arab influence was considerable).

On his before-last return to France, Etienne de Flacourt brought back plants, seeds, and animals from Madagascar. Among these samples was the now famous Madagascan periwinkle which was planted, probably in the form of seeds, in the King's Garden, now known as the Garden of Plants of the National Museum of Natural History in Paris. Like many plants, the periwinkle may have first been subjected to a quarantine in a French Mediterranean or Atlantic port. This general procedure, in fact, explains the present richness and diversity of the botanical gardens in Bordeaux, Nantes, Brest, Le Havre, Marseille, Toulon, etc. Flacourt, however, did not bring back any empirical information concerning the medicinal usage of this plant.

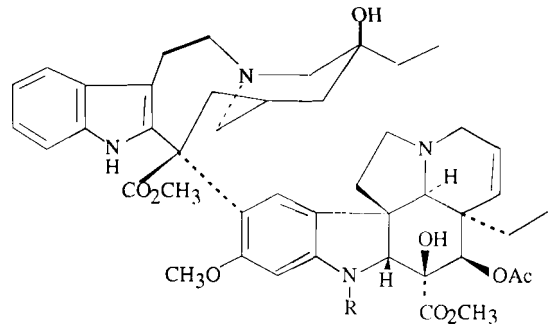
It was already the custom, at that time, that the botanical gardens of the colonizing countries exchanged their collected samples. It is for this reason that *Catharanthus roseus*, the Madagascan periwinkle, reached Chelsea Gardens in London and the Botanical Gardens in Leyden (The Netherlands). From here, the plant was sent to English and Dutch colonies in the West Indies, Indonesia, India, Africa, etc. As a result, the Madagascan periwinkle is now widely disseminated throughout the world where it grows in regions spreading from the equator to the Mediterranean countries.

It was not until 1955 that Canadian scientists, successors of the famous Banting and Best group that discovered insulin at the University of Western Ontario in London (Canada), became by chance interested in the Madagascan periwinkle (Noble *et al.* 1958). This team was looking for substances, natural or synthetic, which could be used to control diabetes. The results of an

ethnopharmacological survey in Costa Rica and in the West Indies indicated that the Madagascan periwinkle was used by the locals to treat diabetes. These Canadian scientists thus prepared an extract of this plant and evaluated its antidiabetic activity in rats rendered diabetic by ingestion of alloxan. Not only was the periwinkle extract found to be inactive, but most of the treated animals died of septicemia. An autopsy of these animals revealed that they were all in a state of leucopenia (a large decrease in the number of white blood cells) which, as everyone knows, leads to collapse of an organism's defenses against microbial and viral infections.

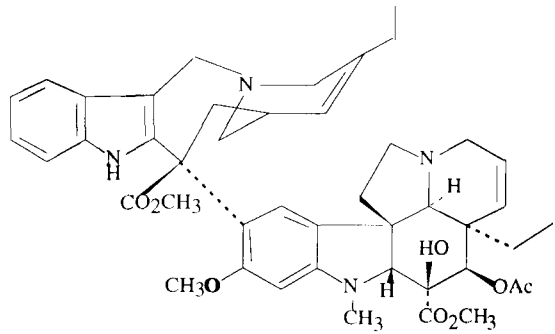
It became immediately evident to these investigators that, if in fact the Madagascan periwinkle extracts were responsible for this leucopenia then it should be possible to isolate the "active principles" and perhaps use them for the treatment of leukemias, a disease characterized by a much higher than normal white blood cell number. It was, in fact, in this manner that the beneficial effects of "alkylating agents" such as "mustard gases", used during the First World War and, unfortunately, more recently by Irakian troops against the Kurds and Iranian soldiers, were discovered. Thus, with the end of hostilities in 1918, it became necessary to destroy remaining stocks of mustard gas. During this destruction, accidental leakage led to serious intoxications and here again, pronounced leucopenia was observed in the victims, whence the idea of using these chemicals to lower the number of white blood cells in leukemia patients.

The reasoning was identical for the Madagascan periwinkle. However, before continuing with this story, it must be added that an unexpected event occurred at this time. On the other side of the border, in the United States, scientists at Eli Lilly in Indianapolis had undertaken systematic screening of plant extracts in their search for compounds displaying antitumor activity (Svoboda and Blake 1975). And this is how they observed that the Madagascan periwinkle contained substances active against several experimental murine tumors (L 1210, P388, etc.). The necessary contacts were established with the Canadian scientists and so began the long years of research which led to the isolation of vincaleukoblastine (vinblastine) and leurocristine (vincristine) eventually commercialized under the names of Velbe® and Oncovin®, respectively. These two drugs have now been used for over thirty years in the chemotherapy of cancers and leukemias. Their complex chemical structures led to some remarkable structural chemistry studies around 1957 in which all of the most sophisticated physical methods available at that time (mass spectrometry, nuclear magnetic resonance and, finally, X-ray crystallography) were used to solve this problem. This type of "secondary metabolite" is still, thirty years later, one of the most complex natural molecules known. Certain toxins from marine organisms as well as some antibiotics have since taken first place for structural complexity, but the vinblastine group of molecules still hold an honorable position !



Vinblastine (R= CH_3), Vincristine (R= CHO)

Another characteristic of these drugs is that they are among the most expensive in our therapeutic arsenal [113.20 FF (*ca.* US$ 22.-) for 10 mg of Velbe® and 85.60 FF (*ca.* US$ 17.-) per mg of Oncovin®]. This is due to the very low concentrations of each drug in the plant (several grams per ton of the dried air-exposed parts of the plant). The synthesis of these types of molecules resisted the numerous onslaughts of chemists for seventeen years. Finally, in 1974, my collaborator, Dr. Nicole Langlois and myself managed to synthesize these molecules by using a "biomimetic" approach (that is one imitating Nature). This success not only allowed us to synthesize most of the compounds of this family known at that time (vincristine, vinblastine, leurosine, leurosidine, etc.) but also, and more importantly, to synthesize a completely original type of substance, Navelbine® (nor-anhydro-vinblastine) (Mangeney *et al.* 1979).



Vinorelbine (Navelbine®)

This compound exhibits therapeutic properties superior to those used up until now, in particular, for the treatment of non-small cell lung cancer (or smoker's cancer) (Vokes *et al.* 1994) and, more recently, of breast cancer. It is altogether

probable that Navelbine's spectrum of activity is even wider, particularly if it is associated with other anticancer chemotherapeutic agents.

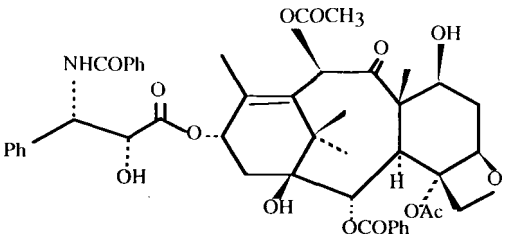
This substance, a product of French public research, is commercialized by Pierre Fabre Laboratories in cooperation with Burroughs-Wellcome (USA) and Kyowa-Hakko (Japan). Navelbine represents a major advance in the area of anticancer chemotherapy ; it will no doubt have successors.

The discovery of Taxotere® (docetaxel) :

I shall now summarize the history of the discovery of another antitumor drug, Taxotere, the fruit, once again, of research conducted at our research Institute at Gif-sur-Yvette in close collaboration with Rhône-Poulenc Rorer research laboratories in Vitry.

The yew (*Taxus* sp., Taxaceae) is a tree well known for its beauty, its longevity, the quality of its wood and also its toxicity. It grows in temperate and sub-temperate countries, resisting well to both cold and heat. The yew is only rarely attacked by insects, fungi or other parasites.

In 1971, Monroe Wall and his group at the Research Triangle Institute in the United States were involved in systematic screening of extracts prepared from plants collected not only in the USA but also from all over the world. This screening program, on a typically huge, American scale, was financed by the National Cancer Institute. Wall discovered that a substance, which he called taxol, present in the trunk bark of the American West Coast yew tree, presented interesting cytotoxic properties (Wani *et al.* 1971). Although he managed to determine the structure of this very complex molecule, his results remained, for reasons I don't understand, for the most part unexploited. One of the reasons is no doubt because taxol could be extracted only from the bark of these multi-centuried trees and that the yield was very low (0.1 to 0.2 g per kg of bark, which corresponds, as we now know, to one therapeutic dose). Since yews are very slow-growing trees, the problem of obtaining sufficient quantities of taxol for testing was an immediate problem.



Taxol®

A second reason is the low solubility of taxol which required the implementation of galenical subterfuges such as the use of "Cremophor", an excipient which allows administrable suspensions of taxol to be made but which is not without its own side-effects.

Several years later, the biological mode of action of taxol was discovered by Susan Horwitz in New York (Schiff *et al.* 1979). Taxol belongs to the group of substances called "spindle poisons". These are compounds which inhibit the formation (or the disappearance) of the spindle during the course of cell division.

Derivatives of vinblastine, whose history I have just evoked, but also colchicine and podophyllotoxine, are spindle poisons. They inhibit the polymerization of tubulin, a ubiquitous protein present in all eucaryotic organisms. This polymerization is the basis for the formation of the mitotic spindle, an event that is essential for cell division. This spindle disappears after cell division has terminated. Taxol, on the other hand, inhibits this disappearance. For this reason, then, taxol is a novel compound.

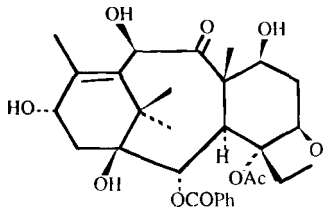
It must be noted that, here again, it is the discovery of a natural product, colchicine, that led to isolation of its biological target, tubulin. The extreme importance of this protein was revealed as a result. The structure of tubulin has not yet been established, though several of these proteins have been cloned.

But let us return to the yew. At our Institute in Gif, our success with Navelbine encouraged us to examine substances which had been described as possessing activity on the tubulin-microtubule (the product of tubulin polymerization) system. This is how our attention was drawn in 1979 to taxol's reported action on tubulin as discovered by Susan Horwitz. The first thing that had to be done was to verify that taxol was present in the trunk bark of the European yew (*Taxus baccata* L.), different from the American yew (*Taxus brevifolia*). This is indeed the case. We were thus able to verify the reported biological properties of taxol, especially with respect to its ability to inhibit depolymerization of the spindle during cell division. However, the major problem which Americans faced, obtaining enough taxol to satisfy therapeutic needs, remained unresolved. The useful therapeutic dose being estimated at 170 mg/patient/month, 2g/patient/year of taxol would be necessary. The number of patients who could benefit from treatment by taxol or better, Taxotere®, as we will see, can be estimated at 250,000. Approximately 500 kg/year of taxol (half as much of Taxotere) would thus have to be made available. Thus, in order to supply sufficient taxol to allow preclinical and clinical studies in the United States, the National Cancer Institute established contracts with West Coast forestry companies to fell 30,000 trees. One can easily imagine the furor of American ecologists on the West Coast, exacerbated by the fact that an endangered species of spotted owl had elected the yew tree as its home !

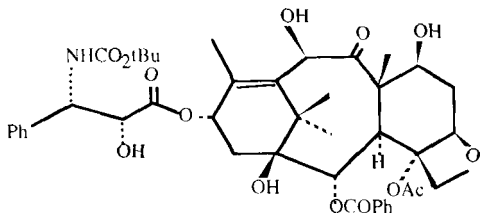
The decision to cut down these trees became, of course, a major topic of the American press. The conservation of nature versus the treatment of patients

suffering, in particular, from ovarian cancer was a dilemma that was widely discussed. All this at a time when it would have sufficed to leave a little room for mathematics : it was quite clear in 1979 when we began studying this problem that if it were necessary to rely on the extraction of the trunk bark of the yew as the sole source of taxol, therapeutic needs would never be satisfied (see above). The complex chemical structure of taxol, moreover, eliminated the possibility of an inexpensive, industrial-scale total synthesis.

It was thus essential to find a reasonable solution to this problem. We thus proceeded to investigate the chemical composition of other parts of the yew. The felling of several of these trees near the C.N.R.S. château at Gif-sur-Yvette, made necessary for the construction of a roadway, furnished us with the raw material needed. It was in this manner that we discovered, in the leaves, a biosynthetic precursor of taxol, that is, 10-deacetylbaccatin III (Chauvière *et al.* 1981), at a concentration of the order of 1 g/kg of leaves (compared to 0.1 - 0.2g/kg of taxol in the trunk bark). It was then simply necessary to do a little chemistry to transform this natural precursor not only into taxol but also into a variety of derivatives. Based on the use of a simple test for biological activity, we were able to select among these derivatives a highly active semi-synthetic compound which we named Taxotere® (Guéritte-Voegelein *et al.* 1990)



10-deacetylbaccatin III



Taxotere®

Taxotere has now been launched in most of the world (except Japan which will come later) for the treatment of lung cancers resistant to other chemotherapies, but also of breast and ovarian cancers.

The two drugs (Navelbine and Taxotere) are synergistic.

It is necessary to insist on an important, but unfortunately still quite rare, occurrence in our country - the shoulder-to-shoulder cooperation between our public laboratory and industrial biology and chemistry laboratories, in this case, Rhône-Poulenc Rorer. Taxotere will, as a result of this collaboration, is now available world-wide except Japan.

Present knowledge indicates that Taxotere constitutes, like Navelbine, another French discovery of an important antitumor drug. Clinical specialists are more and more convinced that taxol and related compounds such as Taxotere constitute the most important discovery in the field of antitumor drugs of the last twenty years. Let us hope so, for the sake of patients afflicted with tumors which do not

respond to other therapies, for instance, certain ovarian tumors for which taxol and Taxotere have been shown to be effective.

Several lessons may be drawn from these successes. The study of natural substances must continue because, despite the considerable progress that has been made in therapeutic research in the design of new drugs, discoveries made by "guided chance" can bring great rewards. The recent discoveries of certain immunomodulators (cyclosporin, FK 506, rapamycin), of inhibitors of cholesterol synthesis (via inhibition of hydroxymethylglutarate CoA-reductase), of antiparasitic substances of the avermectin family, and of many more, are there to attest.

After all, this is not very surprising. The "Creator" has had more than 4.8 billion years to provide us with all kinds of compounds. Much remains to be explored in the garden of Eden. We have contributed a little to this exploration and we will continue despite, as usual, the vaticinations of a few ill-informed people.

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