Global Trends In the Pharmaceutical Industry

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Nature has in the past given us some of our medically and economically most valuable medicines, some from tropical sources, and I am sure she has more in store of us. Puerto Rico´s environment seems to be well suited for such a pursuit. I wish you every success in your endeavor. I have been asked to talk about "Global Trends in the Pharmaceutical Industry". Perhaps the topic has two broad aspects: Trends in "western" science and medical needs – that is : North America Europe and Japan – and the needs of developing countries. Developed countries are interested in the best science and technology to treat ailments of old age effectively, safely and at an affordable cost. Developing countries are struggling with infectious diseases for which medicines don´t exist or are not affordable. It is the new trends in the pharmaceutical sciences that are now most relevant to your plans.

Developing countries are demanding help. AIDS is devastating them. How can we realistically provide support? Let me compare the situation with another tropical disease. In the 1970s, we discovered an antiparasitic drug, the avermectins, that has become a highly effective drug against Onchocerciasis, the so-called River blindness. The drug is dihydro-avermectin Bla/b, Mectisan®. Donated by Merck & Co. to the World Health Organization for as long as it is needed to eradicate the disease. The disease threatens some 80 million people worldwide. A patient needs only one small pill containing 10 mg of the drug once a year. As fewer and fewer people carry the parasite, fewer flies can pick it up and transmit it to other people. Thus, eradication of the disease is now a realistic goal. The drug is now also used for the treatment of Elephantiasis which afflicts hundreds of millions of people in tropical areas.

An infinitely greater logistical challenge is the distribution of a cocktail of anti-AIDS drugs that have to be taken several times a day and must not be shared with friends lest they become ineffective or stimulate the emergence of resistance.

What can the West realistically contribute? It would have to be a vaccine. Intensive efforts are being made to create one, and there is great hope that this will succeed, but it will take a few more years. In the meantime, several Companies have decided to provide their anti-AIDS drugs at a fraction of their cost. Also, Merck and Microsoft have joined forces in an interesting, though small, pilot project which is designed to modernize the overall medical system of one country, Botswana, and to solve the drug distribution problem on a manageable scale in the process. Ten years ago, many Companies started to collaborate with developing countries to save their remaining biodiversity. Perhaps efforts are now underway- a trend, though not yet a global one- to address Third-World medical needs.

With that, let me switch to our main topic. What about modern drug discovery science and the role that natural product screening can play. Succinctly, the question for you is: how can natural product discovery compete with genomics-based approaches and technologies.

During the 19th century, chemists learned to isolate and analyze some of the acive ingredients of medicinal plants and to improve on them. The isolation of salicine and its conversion to aspirin is an example. But no-one knew how these medicines worked. In the 1930s, the mechanism-of-action of the synthetic sulfonamide antibiotics established for the first time a link between the structure of a drug and the structure of an essential component of the biochemistry of a disease. At least in principal, one could now plan to design drugs rationally to resemble molecules that control important biochemical processes.

We knew little about the biochemistry of a disease and had to guess. But gradually it became common practice to select a molecular target and validate its therapeutical significance before starting a drug discovery project, be it by synthetic design or natural product screening. The result has been highly effective and very safe drugs.

Pharmaceutical research is now going through another evolution. The sequence of the human genome has been

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almost completely analyzed. Effective technologies to identify individual genes and their function are being developed at enormous speed. People say it will be another ten years before we really understand the genome. I think we should not be surprised if we get there much faster. Many genes and proteins, that are responsible for now untreatable or poorly treatable diseases, will probably be quickly identified.

At the same time, combinatorial chemistry is becoming a more and more sophisticated science. Let me explain why combinatorial chemistry is so important by comparing its concept with the concept of a structure determination of a new natural product. My teacher in this analytical art, Professor George Buechi at MIT, kept reminding his students that in drawing conclusions from their experiments and spectra they could exclude only those possibilities that they happened to have thought about. Could they be sure that there were no other answers? Then, Professor Carl Djerassi at Stanford University developed a computer program that could write all possible structures that were compatible with a given set of data. I tried this out on the structure of the first carbapenem antibiotic, thienamycin, that we had just solved. The program presented me with a large number of answers, that I had considered and discarded at one time or another, and with one additional possibility, that I had not considered and that at the time was not easy to disprove. The power of combinatorial chemistry methods to synthesize tens of thousands of compounds in a very short time is analogous to Djerassi´s computer program: It allows one to consider in a short span of time not all, but a very large number, of chemical shapes, that one has not explicitly thought about. Some of them will fit the given requirements, such as the binding site of a protein. Although probably no pharmacological factors have been considered at this stage of a drug development, the combinatorial approach to synthesis is a powerful method of discovering and increasing the potency of potential "leads" in much less time than traditional medicinal-chemistry approaches require.

By comparison, the search for natural products is slow. Microorganisms have to be collected and cultured, plants have to be collected and dried, extracts have to be made and chromatographed, structures have to be determined and evaluated. The search is not only slow, you also don't even know whether a compound, that fits your need, exists at all out there.

What then do you have to do to maximize your chances of success? You have to ask some systematic questions and critically examine and optimize every single step of the discovery process. Many questions will come up as you go along. The following are just a few obvious ones, and my comments only sketch a direction we should be going in.

Documentation, sample collection and sample preparation. You don't need voucher specimens between sheets of old newspaper. Documentation can be done by satelite localization, photography and DNA analysis. Instead of bringing just a pound of leaves home, why not collect a quantity in the first place that is sufficient for all planned screeening, a structure determination and developing a cell culture. The pharmaceutical industry has for decades concentrated on microorganisms, in part because they are so convenient to work with. One should work out a similarly convenient way to work with plants - if they really are what one is interested in. Cell cultures could provide the supply for all planned and future screening, provided you can grow them without affecting expression of the biosynthetic pathways of secondary metabolites. If needed, a cell culture can be improved for large scale production of a compound (Taxol can now be produced in huge fermentors).

Extracts contain a lot of material that interferes with assays. Supercritical fluid extraction can select a solubility and molecular size range. The rest can be discarded. The probability that one discards something of value is very small, the gain in time is very large.

Every assay has its characteristic group of recurring "false actives". Knowing them is important - it saves the time that you need to find the "needle in the haystack". Highest priority should therefore be given to isolation and analysis of active components. It can be done on small scale: HPLC, NMR, and MS provide enough information for a substructure search in the literature and quick identification. Biological evaluation of partially purified product only wastes time. The active compound will most likely be a "recurrent false-active" of no further interest. Identify it for future reference and go on.

What type of organism to focus on. Plants have given us important drugs. Quinine, Morphine, Digitalis, Taxol, the Vinca alkaloids - to mention just a few. But I am skeptical of plants as future sources of new pharmaceuticals. Admittedly, science has looked at only a small fraction of all species. But mankind has looked for thousands of years at whatever they could find in their environment, and recent sicentific examination of such traditional medicines has not been productive. The list of 40 or 50 classical medicines, that are often cited in favor of screening of plants, is not convincing. Few would today obtain regulatory approval. I believe plants are extremely important for natural product drug discovery as the scaffolding for what lives on, in and under them in species specific relationships, producing compounds for symbiosis, parasitism, competition. When a plant becomes

extinct, we lose all the associated organisms, that could be much more interesting for drug discovery than the plant itself. We should be interested in ecologies, not just isolated species.

That includes microorganisms of which there are huge numbers that have never before been examined. I would stick with what has in the past been very successful for the Industry, but would try to take a fresh, ecological approach. One would have to avoid species that have been screened many times over by pharmaceutical companies for the last 50 or 60 years, and concentrate on environments that have been largely neglected. Marine microorganisms are an important example.

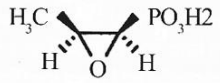
We know little about insects as sources of therapeutics. I believe one of the projects, that NIH's Fogarty Institute sponsors, is investigating them. Traditional medicines, even if their active components are not usable, could conceivably point to new mechanisms-of-action, that we have not thought of. But the effort of analyzing a mechanism is enormous. Genomics offers now a more direct approach to the identification of new targets.

What therapeutic areas to focus on. Generations of chemists have earned their Ph.D. degrees with the isolation and structure determination of natural products. Microbiologists used to call antibiotics "shunt metabolites". Nobody ever seriously asked what the functions of these strange, elaborate compounds are.

Many are probably Nature's chemical warfare agents. This points us in two directions: infectious diseases and cancer. Antibiotic discoveries of the past provide a precedent, and the emergence of resistant strains is a new incentive. Tropical parasitic diseases need urgent attention. Quinine and the avermectins are precedents. Many other scourges of the developing world provide ample incentive. NIH is very successful discovering new cytotoxic agents as potential anticancer drugs. Of course, we hope to find anticancer drugs that are not cytotoxic. But this is perhaps not possible.

There probably exist natural products that can be useful for other therapeutic applications. But if you want to maximize your chances and fill an urgent need, infectious diseases and cancer would seem to be the choice.

What type of screening assay to use. There are sharply focused assays that target single proteins; or "pathway" assays that cover many biochemical steps; or *in vivo* assays. Synthetic compounds must, of course, be tested in single-protein assays. The choice for natural products is not so clear. How do we know what precise mechanism-of–action a microorganism or plant has chosen to defend itself. Our most successful antibiotic screening assay at Merck tested for inhibition of the synthesis of the bacterial cell wall. Dividing cells, that are prevented from rebuilding their wall, will take up water and explode in standard medium. In isotonic medium they would assume a spherical shape, which can be observed under the microscope. It was, of course, not a "very high throughput" assay, but had two advantages: low probability of finding compounds that are toxic to humans (because we don't have tissues that resemble the bacterial cell wall) and the chance of hiting any one of the many biochemical steps of cell wall synthesis. The assay discovered three new antibiotics that all became marketed products*.



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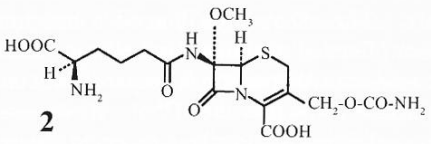

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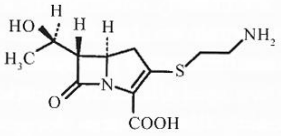

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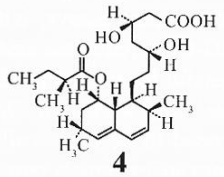
We discovered the cholesterol-lowering drug Mevacor®, the lactone of (**4**), using an assay that tested the inhibition of HMG-CoA-reductase, the enzyme that converts hydroxymethyl glutaric acid to mevalonic acid (**5**). It is the rate-controlling step in the biosynthesis of cholesterol. A closely related HMG-CoA-reductase inhibitor, Compactin, had been discovered at the Sankyo Laboratories in Japan, using an *in vivo* assay, feeding C^{14}-labelled acetate and drug to rabbits. Both Merck and Sankyo were lucky. We could have failed to find anything at all; Sankyo could have found any of the many inferior inhibitors of the pathway that we subsequently observed when we tested other enzymes.



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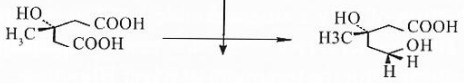

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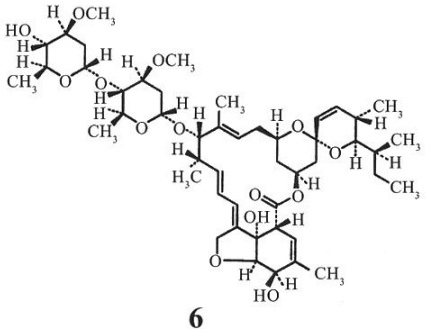
*) Fosfomycin (1), Cephamycin C (2) and Thienamycin (3).

The antiparasitic avermectin (**6**) was also discovered using an *in vivo* assay. Parasitized laboratory animals were fed a diet mixed with lyophilized fermentation broth.



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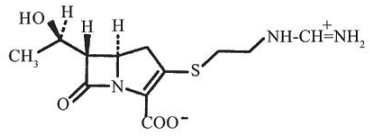
The medicated diet could be either ineffective, ineffective and toxic, effective but toxic, or it could be effective and non-toxic. The assay didn't need a biochemical mechanism-of-action read-out. Oral efficacy and safety were the read-out. Avermectin is very toxic but only if a large overdose spills into the CNS. Its antiparasitic mechanism-of action was totally unexpected and new and is actually still not entirely understood. The compound seems to block a chloride ion channel at the neuro-muscular junctin of the parasite, thereby paralyzing it.

At one time we used the *in vivo* anti-inflammatory assays for screening, that had been designed a long time ago to evaluate corticosteroids. The read-out was reduction of an induced inflammation, with no indication of either a mechanism-of-action or of safety. Not surprisingly, this screening effort was completely unsuccessful.

The three assays that I have just described - the antibacterial cell wall assay, Sankyo's sterol biosynthesis inhibition assay, and the antiparasitic assay - may illustrate some of the best choices of assays for natural product screening. They target entire pathways or novel, unanticipated targets and yet have a meaningful readout. Whether or not you accept an *in vivo* assay, is a personal decision, that may depend on the medical benefit that a resulting new medicine would bring. Countless sharply focused enzyme and receptor assays, with the notable exception of the HMG-CoA-reductase assay, have in our hands not been successful for natural product screening.

What is an "active" and what is a "lead"? Natural products that don't need to be chemically modified to become good medicines are extremely rare. Penicillin and Mevacor® come readily to mind. The avermectins needed the reduction of only a single double bond to maximize safety. The product is Ivomec® for veterinary applications and Mectisan® for the treatment of River Blindness. The carbapenem thienamycin had the potency, breadth of antibacterial spectrum and fascinating structure to make it an immediate lead. It did have considerable stability problems, that had to be solved by first converting an amino group to an amidine (**7**) and then formulating the antibiotic with a specially designed and synthezised inhibitor, Cilastatin (**8**), of a kidney enzyme that degrades the antibiotic; the combined products are Primaxin®.



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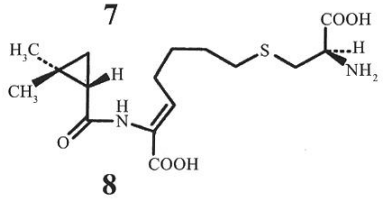

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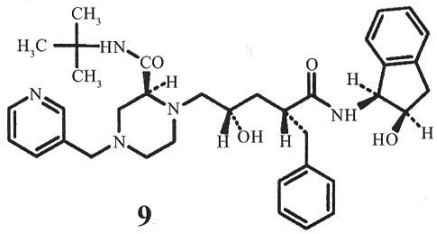
It has been said that all our medicines conceptually go back to some natural product. The roots of the HIV-protease inhibitor Indinavir (**9**) in Pepstatin (**10**) exemplify such a "relationship." Perhaps these examples span extremes. The magnitude of the effort needed to convert a "lead" into a lifesaving drug, is not the most important consideration.

What, then, makes a compound a good lead? A very good criterion is high potency. It was satisfied by all of Merck's natural products that have reached the market. It was satisfied by Indinavir's "conceptural ancestor" Pepstatin. In some cases, potency and structure together are even more convincing: thienamycin's penicillin-like structure, Mevacor's analogy to mevalonic acid. Where applicable, good kinetics of enzyme inhibition or receptor binding are as important or even more important than potency.



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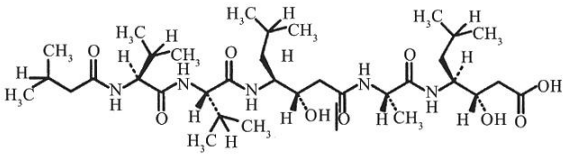

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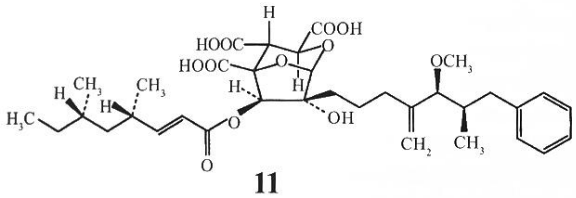
Early *in vitro* studies of pharmacological parameters are important and increasingly employed by the pharmaceutical industry to weed out leads that would fail in the clinic. An example are the Zaragozic Acids (**11**), extremely potent squalene synthase inhibitors whose structures clearly mimic presqualene (**12**). At first sight they looked as interesting as Mevacor, but turned out to be extremely toxic- one more disappointment in an overall extremely successful program.



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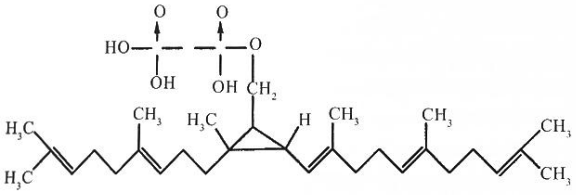

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Conclusion.

I have interpreted "Global Trends in the pharmaceutical Industry" not by describing the trends, but by pointing out what I think they mean for your program and how you might be able to compete with them. The narrow therapeutic focus and old-fashioned assays may seem unattractive to you. To which I can give you two answers:

First, as I mentioned, we have never asked what all those natural products, whose structures have been determined, are doing biochemically. There are some 100,000 in the literature, and only a very small handful are useful medicines. Rather than collecting still other species and adding still other structures to the list, perhaps one should focus on those, that are already known, and subject them to modern screens. This would be far less laborious and much faster. It may be difficult to find reliable samples of some of the compounds, but one could screen species that you have here in Puerto Rico and are related to species that produce known natural products. This may sound a little facetious – but is it so unreasonable?

Let me then close with my second answer: Genomics and proteomics are perhaps not only useful to discover new therapeutic targets, but also to better understand the organisms that are used in a natural product screens, better understand plant and microbial physiology and the biochemistry of symbiosis and parasitism. We are using a universal biochemical process to control cholesterol synthesis in our livers. Are there other universal processes that we could exploit? It could give you clues about which plants or microbes to screen, and in which assay. But I must leave it to you to explore this further.