Natural Products and the Pharmaceutical Industry

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Abstract and Keywords

Natural Products have been exploited by humans as a means of treating illness, ailments and infections throughout recorded human history. Worldwide, NPs are still of major importance in health care. However, there is currently a great debate as to whether NPs will retain their importance as pharmaceutical agents. By the end of the 20th century, all the major pharmaceutical multinational companies had massively reduced investment in NP research. Concurrently, many people interested in international development argued that it was time to increase the study of NP diversity in the less developed countries (bioprospecting) in order to provide novel drugs for the rich, and to provide an income stream for the poor. This chapter shows how an understanding of the evolution of NPs could help transform our approach to finding and exploiting novel pharmaceuticals.

Keywords: Natural Products, NPs, herbalism, pharmaceutical industry, antibiotics, synthetic steroids, anticancer drugs
A drug is that substance which, when injected into a rat, will produce a scientific report.

—Author unknown

Summary
Some NPs have been exploited by humans as a means of treating illness, ailments and infections throughout recorded human history. Worldwide, NPs are still of major importance in health care. However, there is currently a great debate as to whether NPs will retain their importance as pharmaceutical agents. By the end of the twentieth century, all the major pharmaceutical multinational companies had massively reduced investment in NP research. Concurrently, many people interested in international development argued that it was time to increase the study of NP diversity in the less developed countries (bioprospecting) in order to provide novel drugs for the rich and to provide an income stream for the poor. So, where should investments be made in order to increase the chances of finding important new pharmaceutical products? Applying the scientific principles outlined in earlier chapters, it can be shown that an understanding of the evolution of NPs could help transform our approach to finding and exploiting novel pharmaceuticals.

Herbalism
Throughout history, people from all cultures have used herbs and plant extracts to treat illnesses and to dress wounds. There are reports of chimpanzees choosing to eat the leaves of some species that may reduce their gut parasite population. Cats are regularly seen to chew the leaves of grasses, yet ignore other plants (although some cats do get excited by the NPs in catnip). Because of the very powerful placebo effect associated with taste and smells, and because many of the major groups of NPs have strong odours or flavours (see Chapter 8), it is possible that plants and fungi rich in NPs were appealing to some species of animals seeking to improve their health. The increasing intelligence of hominids would have enabled those species to make clearer associations (p.152) between specific ailments and their treatment with NP-containing plants and fungi. Certainly, as soon as recorded history begins, herbalism is clearly well established. We have thousands of years of evidence of the practice of herbalism in many cultures, some hundreds of years of quite detailed treatises on the subject and even in the twenty-first century
the majority of the world's population rely heavily on plant
extracts containing NPs, or purified NPs, for the treatment of
ill health. Indeed, it was only in the twentieth century that the
monopoly of NPs as pharmaceutical products was challenged
by synthetic chemicals. Over four billion people currently use
herbal medicines and even in advanced industrial countries
NP-based medicines are widely used. Nearly 60% of Germans
buy such medicines and the sales of herbal medicines in the
United States were $85 billion in 2007.
The modern pharmaceutical industry starts with a search for a way of making an NP. As described in Chapter 2, in the mid-nineteenth century, the need for greater and more secure supplies of quinine to treat malaria was a challenge that the increasingly confident synthetic chemists were ready to accept. The great race among the newly industrialised European nations for African colonies had begun,¹ and explorers, settlers and the military all needed quinine to enable them to survive infection with the malaria parasite. Although a route to synthetic quinine eluded chemists at that time, Perkin's attempt accidentally led to the discovery of the dye mauve and the birth of the hugely successful dye industry. The growth, and economic importance of the German dye industry in particular, was a major stimulus to the blossoming subject of synthetic chemistry in the late nineteenth century. A very large number of synthetic dyes of all shades and hues were developed and this allowed fashionable colours to change with the seasons—a dominant feature of fashion that remains to this day. The chemical stability and photostability (resistance to fading in sunlight) of the synthetic dyes was essential for their use and some were much more stable than natural vegetable dyes (for reasons discussed in Chapter 4). It was one of these stable dyes, methylene blue, that was to be of particular significance in the establishment of the modern pharmaceutical industry. A young German scientist, Paul Ehrlich, was given the task by the great chemist Hoffman² of trying to establish the path of infection of malaria. Ehrlich found that the methylene blue staining of the parasite in an infected sailor allowed him to trace the protozoan. The parasitic cell had taken up the dye to such an extent that it became visibly stained against a background of cells that were not stained. This suggested that the concentration of dye in cells depended on the cell type. Furthermore, the sailor seemed to recover and showed no ill effects from his exposure to methylene blue. Ehrlich deduced that when many different types of cells were exposed to the same concentration of methylene blue, one cell type must be receiving a high dose of the chemical while another cell type must receive a much lower dose. Given that it had been appreciated for many centuries³ that poisons only acted when they were (p.153) administered above a certain dose, it was reasonable to think that it might be possible to give high (poisonous) doses of a chemical to some cells while leaving other cells unharmed. The idea of selective toxicity was now
based on experimental findings, and Ehrlich was to develop his career with this concept as a focus. It was an exciting time for medical research because it had been established that infectious diseases were caused by an invasion of the body by simple organisms such as bacteria or protozoa. Some of these organisms could be cultured in the laboratory or removed from infected animals and subjected to microscopic study. The small size of these infectious organisms facilitated simple laboratory studies to test whether a particular substance was toxic to the organism. It was practical to ‘screen’ collections of chemicals to find the few chemicals that produced the desired effect at a low dose. The combination of synthetic chemists producing thousands of new chemicals and biologists devising practical ways of testing, cheaply and rapidly, each chemical for some specific biological action was at the heart of the new pharmaceutical industry. However, as discussed in Chapter 5, it was soon realised that a truly selective toxic agent was a very rare chemical indeed. Furthermore, a promising chemical found in a laboratory screening trial often had undesirable side effects when given to an animal. These two problems were to hinder the pharmaceutical industry for the next century, indeed they still hinder it. However, the rewards from finding the very rare truly selective agent were so great that investors found the returns worthwhile and major pharmaceutical companies blossomed in several European countries, in the United States and Japan. Ironically, although Ehrlich started his work studying malaria, a successful treatment for malaria eluded him, and many others, and it took his successors in Germany until 1926 to finally discover the effective synthetic antimalarial drug pamaquine. However, Ehrlich was more successful in finding drugs to treat other infections. In 1905, he showed that trypan red, another dye, was effective at treating sleeping sickness, an infection that debilitated many in the new European colonies. In 1910, Ehrlich discovered Salvarsan as a treatment for syphilis, producing a treatment of interest worldwide to all sections of society.5

Throughout the developed world and throughout the twentieth century, companies were attracted to the huge profits that were available to those who held a patent on a successful pharmaceutical or veterinary agent. In the first half of the twentieth century, the cost of entry into the pharmaceutical or veterinary market was not very high—some simple laboratory
facilities were all that were required for the discovery process. Consequently, nearly all countries with well-developed academic institutions teaching chemistry, medicine and biological subjects spawned small pharmaceutical companies. Few such companies could challenge the dominant German, UK, Japanese, Swiss and US companies but many survived making licensed products, or products out of patent, for local markets. However, as drugs were more widely sold and as medical knowledge increased, it became apparent that even ‘selective’ agents were rarely completely selective. Even if only a small percentage of the population treated showed side effects, drugs could be devastating to individuals. To guard

(p.154)

Table 7.1. The largest pharmaceutical companies and their research and development budgets in 2003. By 2008, the largest Pfizer, had increased their sales to $44,000 million, a sum that exceeds the GDP of over 50% of the world's economies. Four of the companies in the list have merged with others in the subsequent five years.

<table>
<thead>
<tr>
<th>Company</th>
<th>Annual sales (million $)</th>
<th>Annual R &amp; D spend (million $)</th>
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<tbody>
<tr>
<td>Pfizer</td>
<td>28,288</td>
<td>5,176</td>
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<tr>
<td>GlaxoSmithKline</td>
<td>27,060</td>
<td>4,108</td>
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<td>Merck</td>
<td>20,130</td>
<td>3,957</td>
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<tr>
<td>Astra Zeneca</td>
<td>17,841</td>
<td>3,069</td>
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<tr>
<td>Johnson and Johnson</td>
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<td>Aventis</td>
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<td>Bristol-Myers Squibb</td>
<td>14,705</td>
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<tr>
<td>Novartis</td>
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<td>Pharmacia</td>
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<tr>
<td>Wyeth</td>
<td>10,899</td>
<td>2,359</td>
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<tr>
<td>Lilly</td>
<td>10,285</td>
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against highly expensive litigation, pharmaceutical companies had to undertake much more extensive safety testing of newly discovered drugs. Governments also demanded even more data before allowing a drug to be sold commercially. The demands made on companies to gather data to show that drug treatments were both safe and effective massively increased the cost of drug development. These extra costs increased the cost of entry into the industry and forced many smaller companies to merge with other companies. The expensive marketing of branded drugs also became increasingly important. Consequently, the latter half of the twentieth century saw a gradual consolidation of national pharmaceutical companies and eventually a more rapid consolidation into the giant multinational pharmaceutical companies that dominate the industry today (Table 7.1). The largest of these pharma companies are among the industrial giants of the world economy. The combined sales of the pharma companies make this industry the largest legal human activity with current annual sales exceeding $400 billion.

NPs in the pharmaceutical industry—the era of antibiotics
The market for pharmaceutical products is one that will continue to grow because only a small proportion of the world's population currently has access to the most modern (p.155) drugs and as people live longer they need more medical interventions. Significant proportions of pharmaceuticals, or the precursor chemicals used to make them, are NPs from plants or microbes. Some estimates suggest that over 25% of the drugs sold in the developed world and 75% in the low-income countries (LDCs) are based on NPs. Why after more than a century of intensive efforts to make synthetic drugs are NPs still so important? After Ehrlich's success in finding new drugs among the growing collection of synthetic chemicals, it looked for a few decades as if NPs would be eclipsed by synthetic drugs. However, a new golden era was about to begin for NPs, and that era began with successful introduction of penicillin as an antibiotic. Such was the power and selectivity of penicillin that a massive hunt for new microbial NPs began. These searches once again placed NPs back at the centre of drug discovery programmes from the 1950s until the 1970s. Spurred by the dramatic success of penicillin, nearly every large pharmaceutical company in the world started a microbial screening programme in the hope, and expectation, of finding a novel antibiotic. The underlying logic was really economic but there was a scientific justification that could be used to convince any sceptical shareholders. If, as was increasingly believed by many scientists, microbes made antibiotics in order to defend themselves against other microbes, there must be many new antibiotics awaiting discovery; the first to find them could patent them and make a fortune.

Penicillin

The 1945 Nobel Prize for Physiology or Medicine for the discovery of penicillin was shared between the microbiologist Alexander Fleming (who worked at St Mary's Hospital in London), Howard Walter Florey (Professor of Pathology at the University of Oxford) and biochemist Ernst Boris Chain (a member of Florey's team at Oxford University). The story of the discovery of penicillin is as complicated as the characters involved. Most accounts begin in 1928 with the Scot Alexander Fleming (1881-1955) finding by chance that the blue-green mould *Penicillium notatum* secreted a substance that inhibited an adjacent colony of *Staphylococcus aureus*. However, it is now agreed that the young French medical student Ernest
Duchesne has a prior claim as the discoverer. In his 1897 dissertation, Duchesne reported that a *Penicillium* mould contained a potent antibacterial substance. Duchesne partially purified the antibiotic and even carried out a successful assessment of the antibiotic properties of the extract in animals. Unfortunately, Duchesne died at an early age in 1912, but it now appears that Fleming was really rediscovering something that had already been found, even if it was not widely known. In 1929, Fleming published the results of his investigations in the *British Journal of Experimental Pathology*, but he never succeeded in producing enough of the active substance to follow up his early observations and he turned his attention to other lines of study. Significantly, at about that time the first of the effective synthetic antibacterial compounds were exciting interest. The antimicrobial sulfanilamide drug Prontosil was shown in 1935 by G Domagk to be converted in the body to an analogue of the vitamin *p*-aminobenzoic acid and he was awarded a Nobel prize (p.156) for demonstrating its effectiveness against *Streptococcus* and a broad range of other microbes. However, in 1935, the Australian Howard Florey was assembling a team of researchers in the pathology department at Oxford and among those he recruited was the volatile, talented European refugee Ernst Chain. Although notionally recruited to work on cancer, Chain had an interest in Florey's work on the ability of lysozyme (the enzyme found in tears) to kill some bacteria by lysis (breakdown). Chain began reading more about antibiotics and in 1938 he read Fleming’s 1929 paper and it fired his imagination. He repeated Fleming's observations and soon made more progress than Fleming had done. Recognising the significance of the work and stimulated by the thought that in the expected Second World War a large numbers of troops would, as in the First World War, die of bacterial infections, Florey secured government funds to investigate the possibility that Fleming's substance could be a useful antibiotic. Recruiting the modest, but technically imaginative and ever resourceful Norman Heatley to the team, Florey began to culture the mould in increasing quantities despite the limitations due to the outbreak of war. Enough of the substance, soon to be called penicillin, was isolated and partially purified to enable a trial to be made of its effectiveness as an antibiotic on infected mice. Not only did the penicillin cure the infection but the mice showed no significant side effects of the treatment. However, despite
Heatley's best efforts, using bed pans among other containers to grow the mould, the production of penicillin was very limited. At best, only a few milligrams of penicillin per litre of medium was produced, so the typical current dose would have required 200–2000 litres cultured media using Heatley's methods. To compensate, when sufficient penicillin had been accumulated to try on the first patient in the Oxford's John Radcliffe hospital, the patient's urine was re-extracted to glean extra supplies of penicillin to continue the treatment. However, the effectiveness of the antibiotic was so impressive that it was clearly a matter of urgency to find ways of increasing production. The chemical structure of penicillin (Figure 7.1) was being studied to ascertain whether it would be possible to make the chemical synthetically. When the structure was established, the 1000 chemists set to the task of finding a synthetic route were unable to achieve that goal. Fortunately, the ever resourceful Heatley and the determined Florey were sure that the yields of penicillin could be increased from \textit{Penicillium} cultures and improved methods of isolating the substance could make penicillin a practical treatment. Florey decided that a major effort of research and development was urgently needed and he contacted some drug companies in the United Kingdom, and via intermediaries, some of the large US drug companies. Florey had met and made an ally of Lord Rotheschild and through him he met an official in the US drug agency who became Florey's champion in the United States. In July 1941, it was arranged for Florey and Heatley to visit the United States to meet some representatives of the large US drug companies. However, Florey was disappointed and frustrated when he received a rather lukewarm reception from some of these companies. But slowly, and later more rapidly, as the potential of penicillin became clearer to sceptics, a programme was established in some companies to devise methods of mass production of penicillin. One breakthrough came at an (p. 157)
unlikely venue. The Peoria Laboratory in the US midwest had been given the task of finding ways of helping the agricultural economy in the area and was looking for some way of using the huge amounts of corn steep liquor that was a waste product of corn starch production. They had found that it was a very useful fermentation medium and had devised ways of culturing microbes in massive airlift fermentors. Heatley joined (p. 158) the Peoria Laboratory for some months, where he shared his experience with the local experts and soon the group greatly increased the yield of penicillin in the cultures. Part of the project also involved screening new samples of *Penicillium* mould to see whether they could find an isolate which was inherently more efficient at penicillin production. The US military, now establishing new bases worldwide as part of the war effort, were asked to send soil and vegetation samples to the Peoria Laboratory and thousands of samples were evaluated without significant success. Ironically, a technician in the laboratory spotted a nice green mould on a melon in a local Peoria market and when they cultured that local sample it was the most effective penicillin producer of all the samples tested. This local mould became the source of the penicillin as it went into production. By late November 1941, Andrew J Moyer, a Peoria expert on the nutrition of moulds, and Norman Heatley had succeeded in increasing the yields of penicillin 10-fold. More extensive, highly successful clinical trials took place in 1943, and penicillin production was then rapidly scaled up so that supplies were available to treat Allied soldiers wounded on D-Day.

![Figure 7.1. The structures of the major NPs of importance as pharmaceutical drugs.](image-url)
improved production and isolation methods allowed the price to drop from $20 per dose in July 1943 to $0.55 per dose by 1946, a quite remarkable achievement. Current fermentation methods and high-producing strains now make it possible to produce 1000 times the amounts that Heatley could make. Although penicillin was never patented, a fact that caused some friction between Florey and Chain, patents were granted on some of the improved methods of production developed by the Peoria Laboratory and by some of the industrial laboratories that had also become interested. For example, in 1948, Andrew J Morton was granted a patent for a method of the mass production of penicillin.

The discovery of penicillin placed NPs back on the agenda of all the major pharma companies. Improved methods of production were developed and chemically modified penicillin analogues, with improved clinical value, were patented and widely adopted. Such was the optimism engendered by penicillin that it was rashly predicted that bacterial diseases would eventually be eradicated from the human population. Anyone with a reasonable knowledge of evolution and of NPs would have been surprised had that prediction come true.

Why devote so much space to the discovery of penicillin? Simply because penicillin was the first NP to be made in massive amounts in factory scale fermentations, because of its remarkable biomolecular properties. This showed, for the first time, that microbially produced NPs were economically accessible to large populations of humans and that chemists had no monopoly on synthetic methods for the pharmaceutical industry. The story also tells us that a worldwide search for cultures best suited to making penicillin showed that it is the rare organism that makes antibiotics in large amounts, a conclusion confirmed by the next part of the story of antibiotics.

**Streptomycin**

In the late 1930s, another search was underway for microbially derived antibiotics, a quest lead by the Ukrainian immigrant to the United States, soil microbiologist Selman Waksman (p. 159) Waksman of the Department of Soil Chemistry and Bacteriology at Rutgers University in New York. This was a planned programme of screening that eventually led to the discovery of streptomycin from *Streptomyces griseus*. Streptomycin was active against a number of bacterial
diseases and was especially valuable because it was active against some species that were not controlled by penicillin. Streptomycin was effective against tuberculosis (\textit{Mycobacterium tuberculosis}), walking pneumonia (\textit{Klebsiella pneumoniae}), fowl typhoid (\textit{Shigella gallinarum}), one of the bacteria involved in some food poisonings (\textit{Salmonella scottmuleri}) and two bacteria that cause urinary infections (\textit{Brucella abortus} and \textit{Proteus vulgaris}). Selman Waksman was awarded the Nobel Prize for Physiology and Medicine in 1952 for his ‘ingenious, systematic and successful studies of soil microbes that have led to the discovery of streptomycin, the first antibiotic remedy against tuberculosis’.10

Streptomycin is one member of the family of aminoglycoside antibiotics. Members of the family made by strains of \textit{Streptomyces} have names ending with -\textit{mycin} and those made by cultured strains of \textit{Micromonospora} have names ending with -\textit{micin}. These NPs inhibit protein synthesis in various types of bacteria. Unfortunately, some of the family have adverse effects on kidney functioning or hearing in the treated patients; hence these drugs tend to be used as a second line of defence. Bacterial resistance has also become widespread, with several predictable mechanisms recorded. Some resistant strains have evolved with changed mutated proteins on the 3OS ribosomes, proteins that bind the antibiotic less strongly. Other strains take up the antibiotic poorly and some can degrade the antibiotic. The latter form of resistance is due to the production of an enzyme coded for by extrachromosomal DNA that is carried by a plasmid (a small circular piece of DNA that can be passed between bacterial species). This typical example of detailed investigations of the cause of antibiotic resistance development helps to form ideas about the role of antibiotics in evolution, which are discussed in the next chapter.

\textbf{Gramicidin}

In 1939, René Dubos, Waksman's former postdoctoral student, extracted two chemicals, tyrocidine and gramicidin, from the soil germ \textit{Bacillus brevis}. These chemicals cured bacterial infections in cattle but were too toxic for humans. This discovery prompted a number of scientists to expand the search for microbes in the soil, microbes capable of making chemicals that could kill disease-causing bacteria in humans.

NPs in the pharmaceutical industry—the synthetic steroids
Synthetic steroids

By the 1930s, it was clear that some humans suffered from deficiencies of steroids, compounds related to the steroidal NPs found in plants and microbes (see Chapter 9 for the debate about whether some steroids should be classed as NPs). The use of steroids to treat such patients was limited by the supply of steroids which had to be laboriously extracted from animal-derived material and, consequently, were prohibitively expensive. The US chemist Russell Marcker, working at Pennsylvania State University, realised that it should be possible to make human steroids from the diosgenin, the structurally related steroidal compound made by plants. This did indeed prove feasible when it was shown that a steroidal extract of a Mexican plant could be converted to the human female hormone progesterone. Unable to gain interest or support from the US drug companies for his discovery, Marcker found a Mexican businessman ready to invest in a new company, Syntex (Synthesis + Mexico), to exploit this discovery. Sadly, Marcker was swindled of his share of the profits that soon flowed from this company. When he tried to form a rival company, he was subject to physical and legal harassment and maybe wisely retired from industrial chemistry in 1949 and became a dealer in Mexican antiques. However, Marcker had begun an industry that blossomed in subsequent decades as the contraceptive pill, based on plant-derived synthetic steroids, became a major pharmaceutical product and helped women in many countries, both developed and developing, make their own reproductive choices for the first time.

The next great chemist to take up the challenge of making other human steroids was the Austrian-born Carl Djerassi, who fled his country after the Nazi invasion in 1938, and joined the Swiss owned CIBA company in New York. He subsequently joined Syntex (now a respectable company after that shady start) and devised a way of making cortisone from extracts of Mexican yams or sisal. However, the Syntex synthesis of cortisone was never commercially successful because a competing method, involving the use of microbial fermentation, could provide a cheaper product.

NPs in the pharmaceutical industry—the era of anticancer drugs

Vinblastine
One of the most valuable treatments of several forms of leukaemia is the NP vinblastine. Its discovery is yet another example of serendipity. In 1952, the Canadian Dr Robert L Noble (Associate Director of the Collip Medical Research Laboratory at the University of Western Ontario) received an envelope from his brother Dr Clark Noble containing 25 leaves from the Madagascar periwinkle plant (*Catharanthus roseus*). One of Clark Noble's patients in Jamaica had told the doctor that a periwinkle tea was used in Jamaica for diabetes treatment. Dr Robert Noble started an investigation of the properties of extracts of the leaves but he found that there was little effect on blood sugar levels but unexpectedly white blood cell counts had decreased in animals treated with the extract. Given the fact that the uncontrolled production of white blood cells was associated with leukaemia, this finding suggested that a periwinkle leaf extract might be worth investigating as a treatment for leukaemia. In 1954, Dr CT Beer (an Oxford trained organic chemist) joined Dr Noble's research team and by 1958 they had successfully isolated and purified a potent alkaloid extract from the leaves. They named this extract vinblastine. Collaboration with the pharmaceutical company Eli Lilly followed and sufficient vinblastine was produced for clinical trials to begin in 1959 at the Princess Margaret Hospital in Toronto. While not a cure, vinblastine in combination with other drugs was very effective in controlling the growth of a number of different types of cancers. Vinblastine is still one of the most useful chemotherapeutic agents available and its discovery and isolation is considered to be a milestone in the history of cancer chemotherapy, particularly for the management of Hodgkin's disease and testicular cancer.

**Vincristine**

Given Eli Lilly's involvement in the development of vinblastine, it is not surprising that the company funded a team to investigate the other alkaloids in *Catharanthus roseus*. One of the most potent alkaloids was given the name vincristine and approved for drug use in 1963, initially as a treatment of leukaemia. Vincristine acts by binding to the microtubules in the cell, disrupting, among other things, cell division.

Eli Lily currently have vinblastine and vincristine sales that exceed $180 million per annum.

**Taxol**
Because of the success in the 1940s and 1950s of finding the major pharmaceutical agents described above, in 1958 the US National Cancer Institute began possibly the world's largest NP screening programme ever, seeking a chemical that might usefully treat some form of cancer. The selective toxicity sought would have to be extreme because the cells that were the targets were not those of another species but abnormal human cells. The programme to screen hundreds of thousands of extracts containing NPs met with very little success. However, in 1963, the US Forest Service provided a sample of Pacific Yew tree (*Taxus brevifolia*) for extraction and testing. Unlike most plant samples tested in the programme to date, extracts of this tree were found to inhibit cell division. However, progress was slow and it took until 1971 before the compound responsible for the activity, named taxol, was identified and characterised. Taxol, like several other anticancer drugs, binds to microtubules, consequently interfering with cell division. The structural complexity of taxol suggested that a chemical synthesis would be extremely challenging and the difficulty in obtaining sufficient material from the forest trees suggested that there might be no commercial future for the product. However, small-scale studies continued and there was a renewed interest and excitement in 1989 when some women suffering from ovarian cancer responded very well to taxol treatment. These results changed the outlook for taxol. An agreement was signed with the large pharmaceutical company Bristol-Myers Squibb for further development and marketing of taxol and a large investment was made both in the possibility of chemically synthesising the drug or finding better natural sources. The challenge of producing enough taxol by extracting plants was a huge logistical task. Like many NPs, the concentration of taxol in the plant is very small; the bark of Pacific Yew trees contains only 0.02%. Furthermore, the removal of the bark for extraction kills the tree. In order to produce 1 kg of taxol, 3000 trees have to be sacrificed. It was calculated that to treat ovarian cancer with taxol in the United States alone would require the destruction of 75,000 trees per year. If the drug were to be made available worldwide, hundreds of thousands of trees would need to be harvested annually. This alarmed conservationists. The harvesting of the Pacific Yews would inevitably lead to the destruction of other trees and the habitat would be degraded. The forests in question were the home of the Spotted Owl, a species that was considered to be
at risk if the wholesale destruction of the Pacific Yew was allowed. Fortunately, human ingenuity came into play and the pressure was removed from the stocks of the Pacific Yew. An exploration of other *Taxus* species identified the needles of the yew (*Taxus baccata*), a widely grown ornamental shrub, as a source of a chemical structurally related to taxol. This chemical could be converted chemically into a close relative of taxol which was also an effective treatment for ovarian cancer. A huge programme of collecting the clippings of thousands of yew trees annually provided a viable source of the drug. Although synthetic routes to taxol have been reported, none has been successfully brought into commercial production, despite every considerable effort. Likewise, attempts to grow *Taxus* cells in culture have not yielded an alternative commercial source of the chemical.

The story of the discovery of taxol illustrates one of the major problems in seeking pharmaceutical agents among the hundreds of thousands of NPs made by plants. An effective, valuable chemical might be found but a practical, economic source of the chemical might not be. Indeed, a natural source of a very important drug could be very bad news for threatened habitats. How that problem might be resolved is discussed later.

Taxol holds another interesting lesson for us. As in the case of vinblastine or vincristine, it is unlikely that the Pacific Yew made taxol to gain fitness by making an anticancer chemical. It is arguable whether the fitness of plants is reduced by anything similar to cancer in animals. Some individual plants do have clumps of cells made by repeated division, commonly seen as galls, but these structures are usually the result of the invasion of the plant by an insect or a bacterium. There seems to be nothing analogous to the spreading of a cancer as found in animals and the author knows of no example of plant dying due to ‘cancer’. Thus, there was no rational reason to seek anticancer drugs in the Pacific Yew, or indeed in any plant.

The annual total world market for anticancer drugs is currently about $50 billion and is expected to double within a decade.

The future of NPs as pharmaceutical products
The several examples given in the previous section of the extremely valuable NPs used as pharmaceutical agents are regularly used to justify more funding for NP research. Yet, despite the fact that NPs are still so important to the pharmaceutical industry, the collection of samples of NPs for screening for pharmaceutical activity declined over the past two decades. What explains this lack of faith in NPs by the pharma industry? Have the past 20 years of drug discovery been but a temporary phase, when new ideas and new toys distracted attention away from the proven approach of seeking drugs in collections of NPs?

The loss of interest in NPs

There are several factors that have combined to make the screening of collections of NPs look a less attractive way of seeking new pharma products.
**High-throughput screening**

Large-scale screening of NPs is expensive and slow; it generates many false leads and it is intellectually dull. The only intellectual excitement that came to the subject in the 1980s and 1990s came from engineers who designed computer controlled robotic systems to dispense and analyse samples. These robots could not only do the boring work with great precision but they could also record and display the data. Biochemists, working with these engineers, devised biochemical procedures that could be miniaturised so that the enzyme activity in a sample, or the binding of a substance to a particular protein, could be measured in thousands of samples a day with little human effort. This approach became known as high-throughput screening (HTS).

Not only did the HTS robots operate 24 hours a day and 365 days of the year but the HTS approach also capitalised on the rapidly increasing knowledge of cell functioning. Drugs discovered in the middle of the twentieth century had nearly all been found using whole organism screens, with the eventual target of the drug being unknown at the time of discovery. However, as the mode of action of existing drugs was discovered, it was clear that the selective toxicity so essential for use was always based on some fundamental protein–ligand interaction (see Chapter 5) that could be analysed and understood. Could not the traditional approach be reversed? Instead of finding a useful biological action and then understanding the basis for that action, why not use the current knowledge of cell functioning to predict how to find agents that acted on the target process alone? The newly developed methodologies of HTS were ideal for such an approach. HTS depends on seeking a chemical that shows a particular kind of biomolecular activity. This seemed a very great advantage because it optimised the chances of finding the highly selective action that is the dream of all those seeking a new drug. For example, a screen of a collection of chemicals to find an anticancer drug that uses a cell multiplication assay will identify many chemicals that are toxic to some process in the cell and will thus stop the cell dividing —there will be many false positives due to the fact that the cell has many targets for chemicals that act specifically or non-specifically in a toxic manner. However, a screen that is based on microtubule functioning, or better still a specific aspect of
that functioning, will find only chemicals that hit that target. The refined assay will find many fewer false positives.

(p.164) All the major pharma companies invested heavily in HTS in the 1980s and 1990s, but the success of the engineers soon produced a new bottleneck. The capacity of the HTS instruments grew faster than the rate at which new chemicals were being made. The capacity to test samples for the very specific activity grew from hundreds of samples a day to tens of thousands of samples per day. It was soon very apparent that the more specific the target selected for study the lower the frequency of finding any significant activity when the chemicals were tested at low concentration (for the reasons explained in Chapter 5). Given that the cost of conducting every test was now very low, the new limiting factor in drug discovery was the size of the collection of chemicals available to test. Even during the early years of HTS, a library of 10,000 chemicals could be screened within a few days. The challenge switched from how to screen chemicals quickly, cheaply and efficiently to how to increase the size of the library of chemicals that a company possessed.
Combinatorial chemistry

For 150 years chemists had been trained to synthesise and purify new chemicals. The purity of the final product was an indication of the chemist's skill. The chemical agents used to bring about transformations in a synthetic sequence had been developed over the years to be good at producing high yields of the desired product. So most organic chemists working for pharmaceutical companies at the time when HTS procedures were developed were using their skill to make particular structures with a high purity of the final product. The structures being made were ‘designed’ to have properties that experience suggested were appropriate for high biological activity and these chemicals were delivered to those conducting the screen in a purified form. The testing of the activity of the specific chemical was the aim; hence, impurities would just confuse matters. However, there was already a mass of evidence available to show that it was very hard to predict which chemical structure would possess a certain type of biological activity. Experience suggested that after a ‘lead’ had been found (a lead is a chemical that possesses some activity of the desired type) knowledge could be used to make analogues of the ‘lead’ compound, analogues that might be expected to be even better than the original lead. Thus, knowledge was often more useful at optimising an outcome from an initial discovery than it was in finding the original lead. A simple, but very radical, thought emerged from this logic. Why not make as many chemicals as possible, in impure mixtures, and test the mixtures to find the lead and then work backwards to find the active compound once any mixture had been shown to possess the desired, but very rare, biological activity? This approach was to become known as combinatorial chemistry. The aim was to devise synthetic methods that could produce chemical diversity rather than single pure substances.

Molecular biology—another distraction from NP research?

Combinatorial chemists, HTS biochemists and engineers were not alone in taking funds away from those who had for decades being slowly gathering plant samples (p.165) and painstakingly extracting and purifying novel NPs from them for screening. By the 1980s, genes were something to be found in vials, not just in organisms. The techniques and knowledge of the molecular biologists were rapidly assimilated by the pharma companies. Genes code for proteins and proteins were now something that could be made in
fermentation vats at a reasonable price. Insulin and human growth hormone, very valuable substances for patients who cannot make sufficient themselves, were traditionally laboriously extracted from animal organs. By expressing human genes in microbes and using the fermentation methodologies (well known to most pharma companies because of decades of growing microbes for antibiotic production) to grow the genetically transformed microbes, human insulin and growth hormone could be manufactured for the first time. In theory, a company could realistically expect to be able to make any protein of clinical use, either to supplement deficiencies or to be used as a diagnostic tool. Furthermore, because patents could be taken out on novel genes, the discovery of the role of a gene in any form of disease or ailment could be a very valuable asset to a company. Seeking genes associated with appropriate diseases or ailments not only provided potential valuable diagnostic tools, but also opened the possibility of using the expressed proteins in a new HTS methodology to target those proteins.
Bioprospecting—new term for an old approach that attracts new advocates

At a time when the pharma companies were, one by one, reducing their commitment to the search for biologically active NPs in plant and microbial extracts, two quite different groups were advocating the opposite.

Some environmentalists, frustrated by the lack of public concern over the continuing destruction of many important ecosystems, realised that they might have greater success in preserving such ecosystems if they appealed to the self-interest of the public. Clearly, the public valued pharmaceutical products, many of which were NPs. In particular, the most dreaded human illness in the most affluent countries, cancer, seemed to be especially susceptible to treatment with NPs (taxol, vinblastine and vincristine). Put simply, maybe the next important anticancer drug would be found in some obscure plant living in some threatened ecosystem? Hence, there were both economic and humanitarian reasons to preserve such threatened ecosystems because these ecosystems have a high probability of containing organisms that will be able to produce the next generation of NPs needed by humans for drug use. The term bioprospecting was introduced to describe what had traditionally been called NP screening and it is a term that has become widely used by its advocates. The enthusiasm for bioprospecting can be judged by the fact that as of late 2008, 141,000 sites are found when searching Google for this word.

The general public readily picked up these ideas in a simpler form from the popular press. The idea that the next generation of miracle cancer cures awaited discovery in the rain forest, in coral reefs or in the deep ocean, certainly made many people (p.166) take a greater interest in the preservation of these habitats. Furthermore, the arguments in favour of preserving biodiversity in order to retain the value in the chemical diversity gained support from some analyses by academic economists. Balick and Mendelsohn\(^{13}\) studying the harvesting of medicinal plants from a rain forest estimated that annual revenues of $16–61 per ha could be achieved; hence, a high value could be placed on the rain forest for that use alone. Pearce and Puroshothaman\(^{7,14}\) took that analysis further when they estimated that OECD countries might suffer an annual loss of £25 billion if the 60,000 threatened species were actually lost as a medicinal resource. The environmentalists,
and those economists who had calculated the value of these as yet undiscovered chemical resources, were especially encouraged by the fact that there were some examples of bioprospecting in action. Two examples were quoted regularly as evidence of the value of bioprospecting. First, the widely publicised agreement by Merck and Co. to enter into a bioprospecting agreement with the National Institute for Biodiversity (INBio) in Costa Rica in 1991. Second, the investment by Eli Lilly in Shaman Pharmaceuticals, a small company that aimed to use local ethnobotanical knowledge to target plants with a high chance of containing a physiologically active NPs. By the 1990s, it seemed that the pharmaceutical companies were not alone in renewing their interest in screening chemicals from the natural world. The US National Cancer Institute (NCI) had restarted its programme to look at NPs, despite the fact that the previous NCI bioprospecting programme (1955–80) had screened 200,000 extracts for anticancer activity with such limited success that the programme was run down. By 1995, the NCI had produced 40,000 extracts for screening, and out of that 18,000 extracts had been screened for anticancer activity. By that time about 1% showed some positive activity.

This apparent renewed interest in plant products as a source of pharmaceutical leads in the 1990s led optimists in the development community to identify an opportunity to build a revenue stream between the rich, health-conscious, but resource-poor (in biodiversity terms) nations and the poor, resource-rich less developed world. Discussions about bioprospecting moved on to consider issues of equity—how could the poor, developing nations negotiate a good deal with the powerful drug multinationals? How could any income stream that was negotiated be targeted at the most appropriate groups within the developing country (and who were such groups?). Much has been written about these equity issues but less has been written about the logic behind the basic premise that bioprospecting is the best way of discovering drugs. Are rain forests, coral reefs or pristine oceans really a wonderful source of chemical diversity? More importantly, is this chemical diversity likely to contain the next generation of blockbuster drugs?
Among economists, there is still a debate regarding the rewards that can be expected from bioprospecting. Rausser and Small,\textsuperscript{17} after a thorough theoretical analysis, concluded that using the accumulating ecological, ethnobotanical and biological knowledge it should be possible to make the screening of NPs much more rational and hence much more productive.

\textbf{(p.167) Bioprospecting—the reality}
Drug development is more than drug discovery

Rausser and Small overlooked several factors in their economic analysis of bioprospecting and hence overestimated its potential. Four of those factors are crucial and evidence for the importance of those factors was available by looking at the experiences of the big pharmaceutical companies.

The first problem with the analysis of Rausser and Small was that they overemphasised the cost of lead discovery relative to the total cost of bringing a drug to market (now estimated at several $ billions). As discussed earlier, the cost of screening samples has dropped dramatically, with less effort being needed to screen large libraries and improved screening methodologies having reduced the number of false positives being found. The major costs of drug development are now safety testing, preclinical trials and clinical trials. The industry has been seriously alarmed by the fact that very extensive safety testing still does not eliminate the possibility that a drug will reach the mass market before rare adverse side effects start to be recorded when the range and number of patients massively exceeds the number and range that can ever be studied in clinical trials.

Second, Rausser and Small, like many who admire the ethnobotanical knowledge of herbalists, overemphasised the importance of ecological and ethnobotanical knowledge in facilitating the selection of the plants to collect. Although many undeveloped societies possess a very rich cultural knowledge about the use of plants and fungi for medicinal uses, much of that knowledge will relate to diseases or ailments that can already be treated in western society by existing drug treatments, hence there may not be a commercial need to find further treatments. Likewise, many conditions that are a serious concern to western societies, and for which there might be a very great need for a improved drug, might be conditions that the simpler society has never experienced, hence there might be no appropriate ethnobotanical knowledge. The diseases of the rich, overfed, possibly stressed urban westerners are not the diseases of the poor, rural, forest dwellers. The diseases associated with old age are, likewise, likely to be of less interest in communities where most individuals die in childhood. The most striking example of a mismatch between traditional shaman knowledge and the needs of a modern society is HIV/AIDS where a novel emerging disease must be tackled without relying on past
experience. These arguments are not supposed to belittle or undervalue local ethnobotanical knowledge in any way; the arguments are made simply to indicate that some knowledge cannot be expected to guide the large pharma companies that target very different populations.

Third, and most crucially, Rausser and Small failed to appreciate that an active lead is often only useful if a practical, economically appropriate source of that chemical, or a biologically active analogue, is available. This is where synthetic chemicals usually have an advantage over NPs in any drug discovery programme. Self-evidently, a synthetic chemical made for screening purposes must be a chemical that could be manufactured. Although it might be difficult to make chemicals speculatively for screening purposes, the majority of those made and tested are likely to be structures that the chemist was confident could be made with reasonable effort on their part. Such chemicals are likely to be ones that can be synthesised if needed on an industrial scale at an affordable price. This line of argument is not an absolute one but one that relies on a balance of probabilities. Chemicals that are extremely hard to make are likely to be quite rare in libraries of synthetic chemicals. In contrast, when a mixture of NPs is tested in a screening trial, should a potent activity be found, it is quite likely that the isolation, purification and identification of the active principle will be hard and attempts to synthesise the compound, at a cost that can be borne by the market, might never succeed. Would penicillin ever have left the laboratory if it had been made by a microbe that was extremely hard to grow in culture? The examples of taxol or vinblastine also serve to remind us that chemists lag far behind plants in terms of their ability to elaborate some complex chemicals. Thus, a company feeding its HTS with synthetic chemicals can start with a more optimistic and realistic appraisal of the chances of actually being able to bring a product to market than a company feedings its HTS with extracts of NPs.

Fourth, and finally, Rausser and Small seem to have assumed, like so many scientists until recently, that organisms have evolved only to retain biologically active NPs, as if organisms were doing the first stage of a screening trial on behalf of humans. As explained in Chapter 5, the Screening Hypothesis, based on well-established physicochemical principles, postulates that most NPs are simply members of the NP
library that the natural world has made. Like individual chemicals in the libraries of synthetic chemicals made by humans, most of the chemicals will possess no potent biomolecular activity.

To summarise the arguments, the majority of NPs found in plants and microbes are unlikely to possess potent biological activity and even less likely to contain specific, potent biological activity that could be usefully exploited for pharmaceutical use. Furthermore, even when a naturally derived chemical is found to give a good lead, the chemical complexity so characteristic of NPs may make commercial production expensive or impossible. It is surely significant that cultivable microbes have been so important as producers of NPs of pharmaceutical value to humans because they can be selected to overproduce complex molecules that humans would find impossible to make.

Bioprospecting—the future?
Although the earlier discussion explains why in recent years the pharma industry have moved away from NP screening, that does not mean that NPs do not hold great promise as pharmaceutical agents in future. Indeed, the opposite is true. There is a growing acceptance that, as the industry enters the twenty-first century, the expectations of the HTS and combinatorial chemistry era have not been fulfilled. Indeed, the screening (p.169) of hundreds of thousands of chemicals in the 1990s, chemicals made by conventional chemistry and by combinatorial methods using screens for many different targets, has produced an unimpressive list of major new drugs. Quite how bad the problem is cannot be fully quantified; telling the world that you have tested hundreds of thousands of chemicals and have found nothing of value tends to depress your share price and reduce the CEO’s stock option value. However, there is now talk of the need to think again about ways to bring NPs into the screening programmes. The judgement being made is that all synthetic chemicals are all too often very similar to one another. The chemical diversity as drawn in two dimensions on paper, or indeed as represented in three dimensions using computer graphics, does not adequately convey the limited range of ‘pharmacophore space’ that is being accessed by the synthetic structures that are easily made by humans. As explained previously (Chapter 4), humans are good at building up simple carbon skeletons and quite good at building up more complex ones if they put enough effort into the task. Humans are also fairly able to elaborate these skeletons in a limited number of ways. But plants and microbes, using enzymes, can elaborate a much wider range of structures, and make more subtle and delicate elaborations, to produce a bewildering range of complex shapes in a huge range of sizes. Maybe, the 3-D complexity of some NPs is just what is needed to form a stable and high-affinity binding to a particular protein? So, how can one introduce NP diversity back into the screening programmes, without all the negatives discussed previously? Some argue that we simply need to turn the clock back and just put more effort into collecting NP samples and testing them. However, others see a more promising avenue to explore, combining ideas that have already been discussed.

Combinatorial biochemistry
The low frequency of finding biologically active molecules was the incentive to develop both HTS and combinatorial chemistry. As explained in Chapter 5, the goal of generating chemical diversity, using enzymes capable of acting on more than one substrate and possibly producing more than one product, is exactly how plants and microbes have evolved to optimise the generation of chemical diversity. Organisms making NPs make use of combinatorial biochemistry to generate and retain chemical diversity. However, humans cannot access more than a small fraction of that NP diversity because most organisms making NPs occur in limited numbers, many such organisms cannot be cultured and it is hard for humans to find the one NP they could use among the huge numbers and amounts of valueless NPs that humans encounter when they extract an organism. So, how could this bleak situation be changed?

If the Screening Hypothesis (Chapter 5) is valid, it should be possible to enhance the generation of NP chemical diversity of an organism by adding to that organism a gene coding for another NP-making enzymic activity from a different organism (see Chapter 10). It matters not from whence that gene comes, it could be from a plant or microbe or even one of the non-specific enzymes involved in transformations of substances in the human liver for example. Such a genetic manipulation of NP-producing (p.170) organisms to generate new NP diversity has already been reported (see Chapter 10). Consequently, bioprospecting might be carried out on laboratory generated organisms. This laboratory-based bioprospecting will have added advantages. First, it will be possible to use organisms that can be grown easily in the laboratory, organisms that can also be grown in large-scale fermentations. This will immediately address the problem of making any useful chemical once it has been found by screening because the need to make the chemical will have been taken into account at the first stage of the process. Second, this approach opens up a huge untapped potential to investigate and possibly exploit the biochemical potential in microorganisms that currently cannot even be grown in the laboratory, let alone in commercial production. It is considered that only a very small fraction (<10%) of the microbes that exist in soil have ever been grown in isolation and this is hardly through lack of effort. The requirements for each organism are unknown and are likely to remain so.
However, the DNA of such organisms is now accessible; hence, their biochemical capacity can be explored by incorporating parts of their DNA into other organisms that can be cultured. These concepts are already being explored and methodologies have been devised not only to engineer such genetic supplementation but also to screen the biological activity of any new chemicals that are made in an efficient miniaturised HTS process. This is bioprospecting in a new guise and one that is based on a random choice of genes rather than a random screen of chemicals. The genes that give the useful product are just as likely to be found in an insignificant microbe that will never be identified, let alone grown in culture, as in the most beautiful tropical tree. Furthermore, as our knowledge of the way in which DNA sequences influence protein structure and function increases, it will be possible to engineer the biosynthetic ability of organisms to change their spectrum of biosynthetic capabilities, hence the products they can make.

Drug metabolism and NP metabolism

Many animal species, especially herbivores or omnivores, evolved to cope with the presence of chemicals in their diet (see Chapter 6). Although specialist feeders, those that feed exclusively on some food source containing high concentrations of a few NPs, may have evolved to detoxify or become resistant to those substances, generalist seem more likely to use generic mechanisms which simply attempt to keep all NPs below a toxic level. The use of generic mechanisms, which are not selected on the basis of being optimised to reduce the concentration of one specific chemical but which are effective against a wide range of substances, means that when humans take pharmaceutical drugs, or administer veterinary drugs to their domesticated animals, these generic mechanisms will have a significant chance of acting on the administered substance. Some authorities now classify this form of metabolism as xenobiotic metabolism, meaning the metabolism of substances made outwith the organism carrying out the metabolism. However, in such discussions NPs are rarely discussed as a very significant evolutionary driving force that has shaped these metabolic properties. (p.171) It is also common in discussions of xenobiotic metabolism to find that there is an assumption that all xenobiotics must be toxic and that any metabolism of the xenobiotic must reduce the toxic load. Of course the reality is
that most xenobiotics (other than drugs) will possess no significant biomolecular activity (for reasons discussed in Chapter 5) and the degradation of a substance creates one or more new compounds that are equally likely to possess significant biomolecular activity; hence ‘degradation’ is no guarantee of detoxification.  

As predicted in Chapter 6, from the perspective of NP evolution, those studying xenobiotic metabolism report generic mechanisms and they have classified the typical degradative sequence as being in three phases. Phase I, typically changes a substance into something more polar (more water soluble), commonly by the action of one of the common, non-specific P450 enzymes found in many organisms. Phase II involves the ‘conjugation’ (the joining together of two entire entities) of the product of the Phase I reaction with a common substance such as glutathione or glycine, again making a more polar substance. Finally in Phase III, the conjugate is further modified or transported from the cell by specific proteins.

It may be time to refocus the discussions of the metabolism of xenobiotics to place the xenobiotic metabolic properties into the evolutionary perspective outlined in Chapter 9. That would place NP metabolism more clearly into the arena and would possibly help integrate discussions of drug metabolism into the broader biological context.

What does this chapter tell us about the way science works?
The role that serendipity plays in science is often a surprise to non-scientists who sometimes think that science is simply about the power of ‘the scientific method’. Fleming’s chance observation of the mould growing on his contaminated plates (penicillin); the chance that a brother sends a few leaves to a scientist seeking an anticancer drug (vincristine); the chance that a technician finds the best antibiotic producer in a rotting fruit in a local market instead of in thousands of specifically sought soil samples (penicillin); and the multiple chances of individuals with two complementary ideas coming together to solve a problem. It is sometimes said that the most successful scientists are those who observe and exploit the unintended, chance events, rather than focusing on a route already planned with their own thoughts.
The personality of scientists, especially when fame or money enters their lives, suggests that scientist are humans first and scientists second. The fact that so many people involved in antibiotic discovery were treated so badly, or treated others so poorly, shows that Nobel Prize winners are not always noble.

Finally, the bioprospecting saga shows how bandwagons attract passengers with all sorts of motives and these bandwagons take a lot of stopping. Once a bandwagon starts rolling, a few respectable researchers sometimes hitch a ride simply because they need money to advance their work and careers. (p.172)

Notes:
(1.) Excellent popular accounts of the European nations', Japan's and to a lesser extent the United States of America's scramble for colonies is given by Eric Hobsbawn's ‘The Age of Empire’ or Thomas Packenham's ‘The Scramble for Africa’. It is sometimes overlooked that it was only in the latter half of the nineteenth century that national governments became directly involved in colonisation. In the previous three centuries, it was largely the commercial exploitation of NPs that motivated a few Europeans to seek to control events in far off lands (see Chapter 2).

(2.) Hoffman, while employed for a period in London, had taught Perkins, the founder of the UK dye industry. Perkins was actually trying to make the NP quinine when he made the dye mauve!

(3.) The great Paracelsus (Phillip von Hohenheim, 1493–541) is credited with the first clear statement that all chemicals are poisons but every chemical has a non-toxic dose—‘it is the dose that maketh the poison’. Most members of society, many politicians (see Chapter 6) and all parts of the mass media fail to appreciate the importance of this fact, a fact which was later formalised in the Law of Mass Action (see Chapter 5). In the United Kingdom, every year, a government agency publishes a survey of the presence of pesticide residues in food. One has yet to see the following headline to the inevitable story ‘No toxic levels of pesticides found in food’.

(4.) Screening is a word borrowed from other human activities where a method is devised to efficiently separate wanted items from unwanted items. The sieve or screen can separate the
grain from the straw or the stones from the soil; in both cases, the grid in the sieve or screen selects on the basis of size. Gold prospectors use a pan to ‘screen’ their material using density as the criteria for selection—prospecting for gold is very similar to prospecting for pharmaceutical drugs in that the majority of material handled is valueless but the rare nugget can be worth a fortune or it might be ‘fools gold’.

(5.) Until that time the main treatment for syphilis had been a prolonged treatment with the highly toxic and cumulative heavy metal mercury—‘one night with Venus and a lifetime with Mercury’.

(6.) The term ‘pharmaceutical company’ has gradually been replaced by the term ‘pharma’ in financial and trade circles.


(9.) This discovery provoked a large programme of the screening of sulphanilamide analogues and this programme provides further evidence that the probability that any substance possessing potent biomolecular activity is low. Around 5488 derivatives of sulphanilamide had been investigated by 1945, yet none could compete with penicillin.
As in the case of the discovery of penicillin, where some believe that the Nobel Committee did not give sufficient credit to the contribution of Norman Heatley, so Waksman has also been accused of giving insufficient credit to his graduate student Albert Schatz. Waksman’s screening programme was initially undertaken by graduate students among whom was Albert, who started work in June 1942. Schatz was drafted into the army in November 1942 and was posted as a laboratory technician to a Medical Detachment of the Air Corps in Florida. He began a search for antibiotics that would be useful against bacterial diseases that were not susceptible to penicillin. However, he was discharged due to ill health and returned as a research assistant to Waksman but continuing the work he had begun in the army. Waksman was taking an interest in *Mycobacterium tuberculosis* and because the bacterium was so virulent, he found Schatz an isolated laboratory in the basement where he was encouraged to screen for antibiotics that would control the pathogen. It was Schatz who was fortunate in isolating an actinomycete, *Actinomyces griseus* (since renamed *Streptomyces griseus*) which produced a substance that was effective against a wide range of pathogens, including *M. tuberculosis*. Schatz received his PhD for his discovery of streptomycin and was the first author of the paper (by Schatz, Bugie and Waksman) announcing the discovery in 1944. (In a sworn affidavit, Bugie later credited the discovery to Schatz and Waksman and suggested that her contribution was minor. However, when the controversial royalty settlement was agreed, Bugie received a 0.2% share. Bugie later in life told her daughter ‘They approached me privately and said, some day you will get married and have a family and its not important that your name be on the patent’—information from Drs. Milton Wainwright and Ross M Tucker.) Before Schatz left Rutgers, a patent was awarded in 1948 to Waksman and Schatz for streptomycin and a gentlemen’s agreement was made that neither individual would profit from this discovery instead all royalties would go to the Rutgers Research Foundation. However, in 1949, problems began and the amicable relationship between the two took a turn for the worst. Schatz, now working at Hopkins Marine Station in California, received documents from Waksman asking Schatz to sign away his rights to credit and any royalties from streptomycin. Schatz learnt that Waksman had negotiated a contract with Rutgers to receive 20% of the royalties from streptomycin which by
that time amounted to $350,000. Eventually, Schatz sued Waksman and Rutgers to the great embarrassment of all. However, rather than going to trial, a deal was reached between the parties where Schatz was to be given credit as the co-discoverer of streptomycin as well as 3% of the royalties. Waksman received 10%, while Rutgers received the lion's share of 80%. The remaining 7% was distributed to all of the students and researchers who participated in the discovery. However, poor Schatz had further reason to feel aggrieved. His lawyer took 40% of his first $125,000 royalty and he was shunned by the scientific community because he had dared challenge authority. Despite the legal recognition of his contribution, Schatz still received no recognition from the Nobel Committee when Waksman was awarded the prize alone in 1952. It took decades before Schatz was given the credit he fought for and even Rutgers finally accepted his contribution by awarding him the Rutgers Medal as co-discover of streptomycin.

(11.) A patent in the name of CT Beer, JH Cutts (a doctoral student and co-worker) and RL Noble was administered by the University of Western Ontario in cooperation with the Eli Lilly Co. of Indianapolis. While Dr Noble has received broad recognition for this important work, Dr Beer's essential role in the vinblastine story has been largely overlooked.


(15.) When the much publicised case of the Merck investment in INBio is looked at more closely, it was clearly a much less enthusiastic endorsement by that company of the potential of bioprospecting. The Merck and Co. investment of $1 million in INBio was less than 0.1% of that company's Research and
Development (R&D) budget in 1991. To put the figure of $1 million into perspective, in 1999 Merck & Co.'s sales were $13,693 million, their R&D budget was $1,821 million and one drug (Vioxx) had potential annual sales of $2,000 million. As the twenty-first century began, the US pharmaceutical industry was spending >$8,000 million on advertising and drug promotion alone. The investments by Merck & Co. in INBio and the Eli Lilly investment in Shaman Pharmaceuticals (which is no longer trading) were crumbs from the rich man's table not serious commitments to NP screening.


(19.) Vioxx, a drug with $1.8 billion US sales in 2003, which was specifically designed to target only a cox-2, has been withdrawn by Merck and Co. after it was reported that some patients had died from side effects. A few individuals sued Merck & Co. for hundreds of millions of dollars—hundreds of times what Merck & Co. invested in INBio.


(22.) The generation of a toxic substance from an innocuous one is known. Such 'lethal synthesis' has been reported in both the pesticide and pharmaceutical industries.