Chimia 54 (2000) 652–657

ISSN 0009–4293

Search for New Lead Compounds from Higher Plants

Kurt Hostettmann* and Christian Terreaux

Abstract: Higher plants represent a rich source of new molecules with pharmacological properties, which are lead compounds for the development of new drugs. During the last decades, the renewed interest in investigating natural products has led to the advent of several important drugs, such as the anticancer substances vinblastine, vincristine and taxol, or the antimalarial agent artemisinin. Success in natural products reasearch is conditioned by a careful plant selection, based on various criteria such as chemotaxonomic data, information from traditional medicine, field observations or even random collection.One main strategy in the isolation of new leads consists of the so-called bioactivity-guided isolation, in which pharmacological or biological assays are used to target the isolation of bioactive compounds. One major drawback of this strategy is the frequent isolation of known metabolites. Therefore, hyphenated techniques (LC-UV/DAD, LC-MS, LC-NMR) have been developed, in order to detect as early as possible potential original structures. These compounds can then be tested in various bioassays. Using a combination of hyphenated techniques and bioactivity-guided isolation procedures, a series of new diterpenoic antifungal quinones have been isolated from the African tree, *Bobgunnia madagascariensis* (Leguminosae) and recently patented for their strong activity and for their potential use in the treatment of systemic mycoses.

Keywords: Antifungal agents · Bioactivity-guided isolation · *Bobgunnia madagascariensis* · Hyphenated techniques · LC/MS · LC/NMR · Natural products · Pharmaceutical chemistry · Plant metabolites

1. Introduction

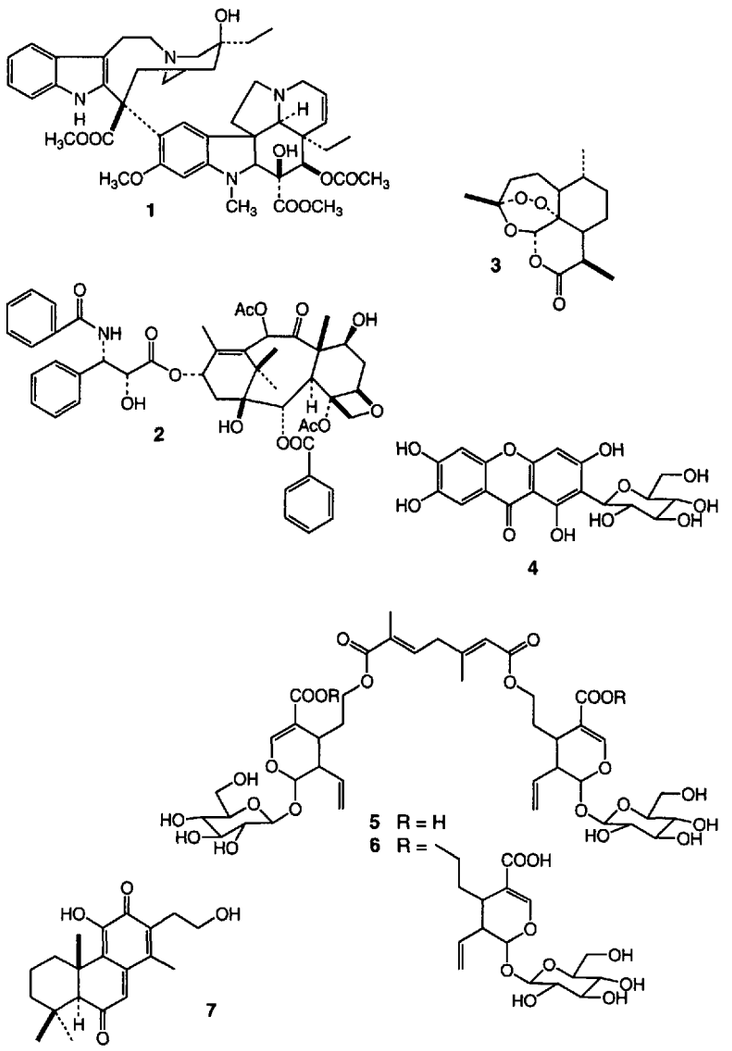
Higher plants represent a rich source of natural products, with an almost infinite molecular diversity. These molecules often have specific functions, but many of them possess pharmacological properties which can be of use to humans. They also may provide lead compounds for the development of new drugs or they may act as indispensable tools in biomedical research. Their role as plant secondary metabolites is not always fully elucidated, but many are substrates for life processes, or are toxins for self-defence, hormones, and molecules with other functions. An estimate of the current interest in natural products is provided by the attempts of the pharmaceutical industry to introduce them into drug discovery programs. With the introduction of high-throughput screening strategies, there is a potential to test large numbers of plant extracts or compounds in a wide variety of bioassays. Since plants are synthesisers able to produce an unpredictable range of skeletal types and novel substances, it is of prime importance to evaluate as many natural products as possible in order to find sources of new drugs or lead compounds.

2. The Importance of Medicinal Plants

Over centuries, the world's populations have used exclusively medicinal plants as therapeutic agents. Nowadays, in spite of the exponential development of synthetic pharmaceutical chemistry, including combinatorial chemistry and microbial fermentation, 25% of prescribed medicines in industrialised countries are of plant origin. This percentage can reach 50% for the OTC market (drugs for self-medication). In fact, it is also estimated that natural products are implicated in the development of 44% of all new drugs, most of the time as leads for the preparation of semi-synthetic derivatives. Over the last decade, there has been a renewed interest in plants and the pharmaceutical industry now considers plants to be full alternatives for the discovery of new leads. Among the estimated 400 000 plant species on the earth, only a small percentage has been phytochemically investigated and the fraction submitted to biological or pharmacological screening is even smaller. Moreover, a plant extract may contain several thousand different secondary metabolites and any phytochemical investigation of a given plant will reveal only a narrow spectrum of its constituents. The plant kingdom thus represents an enormous reservoir of pharmacologically valuable molecules to be discovered [1][2].

For the past thirty years, the emphasis in natural product research has been in the field of anticancer drugs. Thus, in the United States of America, more than 60% of the approved anticancer drugs between 1983 and 1994 were of natural origin [3]. Among them, several were secondary

*Correspondence: Prof. K. Hostettmann
Institut de Pharmacognosie et Phytochimie
Université de Lausanne
BEP
CH-1015 Lausanne
Tel.: +41 21 692 45 61
Fax: +41 21 692 45 65
E-Mail: kurt.hostettmann@ipp.unil.ch



plant metabolites or molecules derived from plant constituents [4]. The alkaloids vinblastine (**1**) and vincristine from the Madagascar periwinkle (*Catharanthus roseus*, Apocynaceae), known since the 1960s are still used in the treatment of leukaemia. The semi-synthetic vinorelbine (3,5-nor-anhydrovinblastine, Navelbine®), has been recently developed for the treatment of breast cancer. The diterpenoid paclitaxel (**2**), known as Taxol®, was isolated for the first time from the stem bark of the Pacific yew *Taxus brevifolia* (Taxaceae) in the late 1960s, but has since been found in other yew species such as the European yew, *T. baccata*. Taxol® was not marketed until 1992, due to supply problems as paclitaxel is produced in the bark of the yew tree. This problem has been overcome by the obtention of paclitaxel through semi-synthesis from 10-deacetylbaccatin II, found in the needles of *T. baccata*. This strategy has also provided access to structural analogues, one of which, docetaxel (Taxotère®), has been recently commercialised. The monoterpenoid alkaloid camptothecin was also first isolated in the late 1960s from the Chinese ornamental tree *Camptotheca acuminata* (Nyssaceae). However, its high toxicity stopped further clinical developments. Considerable efforts have since been made to find more active and less toxic structural analogues. In 1996, two semi-synthetic camptotecin derivatives, topotecan and irinotecan, were introduced for the treatment of advanced ovarian cancers and of metastatic cancer of the colon or rectum, respectively.

In the last decade, natural product research also focused on the development of new antimalarial agents as illustrated by the discovery and development of artemisinin (**3**) [5]. Artemisinin, a sesquiterpene lactone containing an endoperoxide group, was isolated in 1972 by Chinese scientists from qinghao (*Artemisia annua*, Asteraceae), a plant used for over 2000 years in China as a febrifuge and for the treatment of malaria. It represents a completely new chemical class of antimalarial compounds and shows high activity against resistant *Plasmodium* strains. In order to overcome the problems arising from the highly lipophilic nature of artemisinin, a series of derivatives including ethers and carbonates have been synthesised. Among them, artemether, arteether and sodium artesuanate are being licensed as drugs in an increasing number of countries. Despite the new developments, resistance is still present and multidrug associations are now used, such as the concomitant administration of artemisinin derivatives and lumefantrine, a highly active aminoalcohol [6].

Plants are not only a source of pure, chemically defined active principles. They may also be used as extracts, particularly when plants exhibit weaker and/or less specific pharmacological activities or if the active principles are as yet unknown. Moreover, the therapeutic effect may result from the additive action of several active principles. Thus, therapeutic efficacy is strongly depending on the quality of the extract, in terms of active principles' content. Over the last few years, many clinical studies have been published [7] on the therapeutic potential of various plant extracts, including ginkgo (*Ginkgo biloba*, Ginkgoaceae), St. John's Wort (*Hypericum perforatum*, Guttiferae), ginseng (*Panax ginseng*, Araliaceae), garlic (*Allium sativum*, Liliaceae) or hawthorn (*Crataegus monogyna*, Rosaceae). Therefore, such preparations have become widely accepted as therapeutic options in most western countries.

3. Approaches for the Discovery of New Drugs from Higher Plants

Searching for new drugs in plants implies a screening of the extracts for the presence of novel compounds and an investigation of their biological activities. Suspected novel or bioactive compounds are generally isolated in order to proceed to structure elucidation and to perform further biological and toxicological testing (Fig. 1). The path which leads from the intact plant to its pure constituents is long. It involves work which might last from weeks to years and includes the following steps:

- Collection of the plant material with precautions to avoid artefact formation
- Correct identification of the species by a botanist

- Extraction procedure using different solvents, followed by the analysis of these extracts by different chromatographic methods
- Fractionation and isolation steps using different preparative chromatographic techniques (column chromatography, centrifugal partition chromatography, *etc.*)
- Structure elucidation of the constituents by combination of various spectroscopic (UV/VIS, IR, carbon and proton NMR, MS, X-ray diffraction) and chemical methods
- Pharmacological and toxicological testing
- Synthesis or semi-synthesis of the natural product
- Synthesis of analogues with the aim of establishing structure-activity relationships

3.1. *Plant Selection*

The phytochemical investigation of medicinal plants with the aim of isolating and identifying some active substances requires a correct choice of vegetable material. As a large number of plants have not yet been investigated from either a phytochemical or a pharmacological point of view, this choice is difficult. The following criteria can be considered:

- Chemotaxonomic criteria
- Information from traditional medicine
- Field observations
- Random collection
- Endemicity and degree of investigation of the plant

Chemotaxonomy, or the science of classification of plants as a function of the structures of their chemical constituents, can introduce useful elements. Constituents are often produced by metabolic pathways specific to a given botanical family, to a genus or to a species. Thus, if a natural compound or class of natural compounds possess relevant properties, analogous substances may be found in parent species. For example, the gentian family (Gentianaceae) (*ca.* 1100 different species) is characterised by the presence in its roots and leaves of iridoids and secoiridoids, bitter principles stimulating digestion, and of xanthones with antidepressive properties. Then, an investigator interested in the search for new antidepressive agents will select different gentians in order to increase his chances of discovering new xanthones. Chemotaxonomic information can be obtained before beginning a phytochemical and pharmacological investigation by a literature or data bank search; it is thus possible to gain access to all the previous research which has been performed on the plant selected for study. The distribution of xanthones is not restricted to gentians. These interesting molecules are also found in the Polygalaceae and Guttiferae (*Hypericum perforatum*, for example) families [8]. They are absent from most other plant families.

Selection of plants based on *data from traditional medicine* can also lead to promising new molecules. Plants from tropical and subtropical regions represent an enormous reservoir of new molecules with potential therapeutic activity waiting to be discovered. The pharmaceutical industry is aware of this potential and have introduced screening programmes for plants from tropical regions. International conventions have been drawn up in order to prevent an excessive exploitation of these natural resources from Third World countries. Traditional healers in these countries are only accessible with major difficulties and meetings with European researchers are planned in the presence of local representatives (university staff, botanists, *etc.*) in order to discuss medicinal plants, their identification and use. How can the information gathered from traditional healers be evaluated? For example, the efficacy of plants used as laxative or externally to treat sores or wounds are of great interest because their healing effect is easy to verify. However, diagnosis by the healers of internal problems is much more difficult to rely on. Their treatment is very often symptomatic and, moreover, traditional healers often call upon supernatural forces. The placebo effect is also often involved. Therefore, an evaluation of the obtained information is required before selecting plants for further investigation.

During plant collecting expeditions, *field observations* are obviously very important. A plant species growing in a hostile environment, such as warm and humid tropical forests, will attempt to protect itself by synthesising insecticidal, fungicidal, antibacterial or virucidal constituents. Then, for example, if the leaves of a plant show no signs of aggression, they may contain defensive compounds against insects or microorganisms. Roots often biosynthesise antifungal substances because soil is rich in pathogenic fungi. These compounds may also have an antifungal effect against human pathogenic fungi. Such an example will be discussed later with the *antifungal constituents from Bobgunnia madagascariensis (Leguminosae)*.

Random collection is also indispensable. In this case, preference will be given to *endemic* plants which have not been investigated. As endemicity indicates a very narrow distribution of a species, the plant may produce secondary metabolites specific to the bio- and ecosytems where it is growing.

Given the potential of plants which have not yet been investigated and the need for new and effective agents against some diseases (*e.g.* AIDS, multiple sclerosis, Parkinson's disease, Alzheimer disease, certain cancers, *etc.*), additional efforts need to be taken for the discovery of novel therapeutic agents from higher plants.

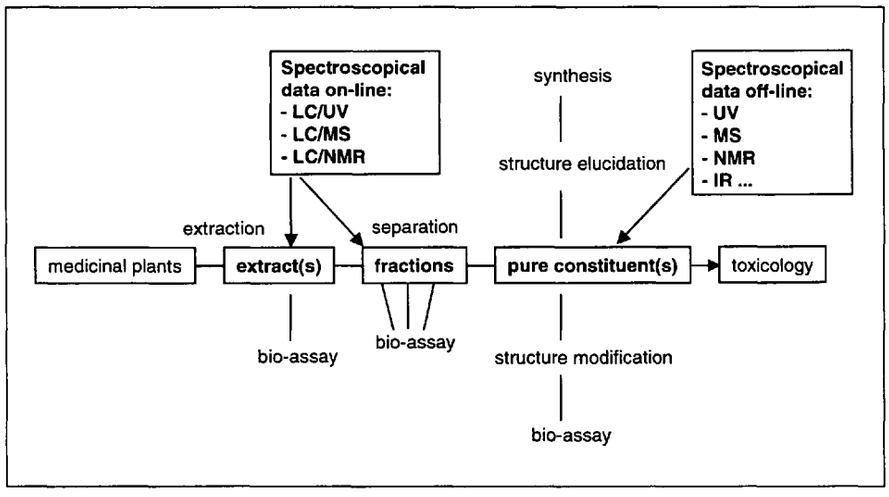


Fig. 1. Sources of therapeutic agents.

3.2. *Biological and Pharmacological Targets*

It is obviously impossible to isolate all the constituents of a plant in order to identify the active principles. Among the different substances, one or only a few may be responsible for the therapeutic or

the toxic effect. Therefore, it is necessary to have access to relatively simple biological or pharmacological tests in order to localise the chosen activity in the plant extracts or in the fractions resulting from the different purification steps which lead to the pure active constituents. These tests have to be very sensitive because the active substances may be present in the plant in very low concentrations. They are also required to be specific for the target involved. The principal targets for biological tests can be divided into six groups:

- Lower organisms: microorganisms (bacteria, fungi, viruses)
- Invertebrates: insects, crustaceans, molluscs
- Isolated subcellular systems: enzymes, receptors
- Animal or human cell cultures
- Isolated organs of vertebrates
- Whole animals

Screening for antifungal or antibacterial activity is a relatively simple process. For example, a plant extract or an isolated substance is placed in contact with pathogenic fungi. It is then straightforward to observe inhibition of spore growth or their death. Certain plants have insect repellent or insecticidal properties, while others are active against insect larvae or molluscs (molluscicides). Screening tests for these activities on invertebrates are also simple to perform. Insecticidal or larvicidal plants can play an important role in the prevention of tropical parasitic diseases, like malaria or yellow fever, transmitted by mosquitoes. As for molluscicidal plants, they can stop the propagation of schistosomiasis (bilharzia), a parasitic disease with a mollusc (freshwater snail) as intermediate host, which affects over 250 million people in Third World countries.

The recent spectacular developments in cellular biology and molecular pharmacology are of prime importance for biological and pharmacological assays based on mechanisms of action. When the molecular basis of a disease is known, it is possible to perform tests with the receptors or enzymes implicated in the disease. For example, substances which inhibit cyclo-oxygenase or 5-lipoxygenase, enzymes involved in the process of inflammation, represent potential new anti-inflammatory agents. Another example is in the fight against cancer, for which inhibitors of the enzymes topoisomerase I and II and protein kinase C, as well as substances which act on the polymerisation of tubulin, are interesting targets. Recently, the coupling of a separation technique (HPLC) and enzymatic bioassays has been realised in order to find new acetylcholinesterase inhibitors from plants. Such active metabolites are particularly relevant in the pharmacological management of Alzheimer disease [9]. Development of similar on-line bioassays represent a new challenge for the future as such techniques, in combination with other hyphenated methods [10], allow an early detection of new bioactive constituents in a plant extract.

Other *in vitro* tests are made on cell cultures. These are also of great importance in cancer research. One of the basic tests is to find cytotoxic molecules or growth inhibitors for tumour cell cultures of human origin. In this particular case, the tested substances have to be highly specific as they are very often toxic to normal cells or do not reach the target tumour. Sometimes, tests on cell cultures are replaced by investigations on isolated organs of animals. Pharmacological models such as the perfused frog heart have been used for the study of cardiac glycosides. Other tests are carried out on the perfused liver, guinea pig heart, isolated chicken veins *etc.* The information provided by these tests is often useful but hardly gives any indication of the mode of action of the sample and cannot be extrapolated to the human situation.

Finally, testing on live animals still takes up a large place in the development of new therapeutic agents, even though enormous efforts are being made to find substitutes for this procedure.

3.3. *Hyphenated Techniques as a Tool to Investigate Molecular Diversity*

One major drawback of the bioassay-guided fractionation strategy is the frequent isolation of known metabolites. Chemical screening of crude plant extracts therefore constitutes an efficient complementary approach, allowing localisation and targeted isolation of new types of constituents with potential activities. This procedure also enables recognition of known metabolites at the earliest stage of separation, thus avoiding costly and time-consuming isolation of common constituents. The potential of the chemical screening strategy has been considerably increased by the recent development of hyphenated techniques, which are able to provide valuable structural information on-line.

HPLC coupled with UV photodiode array detection (LC/UV) has been used for more than a decade by phytochemists in the screening of plant extracts [11] and is now widely employed in many laboratories. The UV spectra of natural products give useful information on the type of constituents and also, as is the case for polyphenols, information on the oxidation pattern. New instruments allow the recording of UV spectra of reference compounds in databases and computer matching can be realised automatically when screening for known constituents.

HPLC coupled to mass spectrometry (LC/MS) has been introduced recently and is still not very common in the phytochemical community [12]. The coupling between LC and MS has not been straightforward since the normal operating conditions of a mass spectrometer (high vacuum, high temperatures, gas-phase operation, and low flow rates) are diametrically opposed to those used in high-performance liquid chromatography (HPLC), namely liquid-phase operation, high pressures, high flow rates, and relatively low temperatures. On-line coupling of these instrumental techniques has been performed with the use of different LC/MS interfaces. Each of these interfaces has its own characteristics and range of applications and several of them are suitable for the analysis of plant secondary metabolites. For the HPLC screening of crude plant extracts, four interfaces, thermospray (TSP), continuous flow FAB (CF-FAB), electrospray (ES) and atmospheric pressure chemical ionisation (APCI) have been investigated [13]. LC-UV-MS analysis of *Gentiana rhodantha* (Gentianaceae) is an example of the on-line detection of original molecules. LC-TSP-MS analysis of the methanolic extract of *G. rhodantha* showed the presence of the predominant xanthone C-glycoside mangiferin (**4**) and different secoiridoids (Fig. 2A). The secoiridoids were eluted with retention times lower than ten minutes and some were easily identified with the help of the LC-TSP-MS spectra recorded on-line as swertiamarin (MW: 374), kingiside (MW: 404), *epi*-kingiside (MW: 404) and sweroside (MW: 358). The slower running peaks (**5** and **6**) also exhibited the characteristic UV spectra of secoiridoids (one band at around 240 nm) (Fig. 2A). The LC-TSP-MS analysis of **5** and **6** gave in each case a spectrum identical to that obtained for sweroside; all exhibited an intense ion at 359 amu and no ion at higher masses. However, the chromatographic behaviours of **5**, **6** and sweroside were quite different. In order to obtain complementary information on these constituents, a second LC/MS analysis with CF-FAB was achieved, using the same

HPLC conditions (Fig. 2B). The total ion current recorded for the whole chromatogram showed a very important MS response for compounds **5** and **6** while the more polar metabolites were only weakly ionised. The FAB spectrum of **5** recorded on-line exhibited a very intense pseudo-molecular ion $[M-H]^-$ at *m/z* 913 together with a weak ion at *m/z* 555 corresponding to the loss of a 'sweroside-like' unit $[M+H-358]^+$. According to the different results obtained on-line in the HPLC screening of the extract of *G. rhodantha,* it could be concluded that **5** was probably a type of moderately polar large secoiridoid containing at least one unit very similar to sweroside. Similar data were obtained for compound **6**, indicating a molecular weight of 1628. Following the LC/MS screening results, a targeted isolation of **5** and **6** was undertaken. A full structure determination of **5** showed that it consisted of two secoiridoid units linked together with a monoterpene unit through two ester groups [13]. The full structure determination of **6** showed that this compound possessed two more secoiridoid units. These two compounds were found to be natural products of a new type [14]. This example illustrates well the use of LC/MS for investigating molecular diversity of a plant and thus targeting the isolation of unknown compounds.

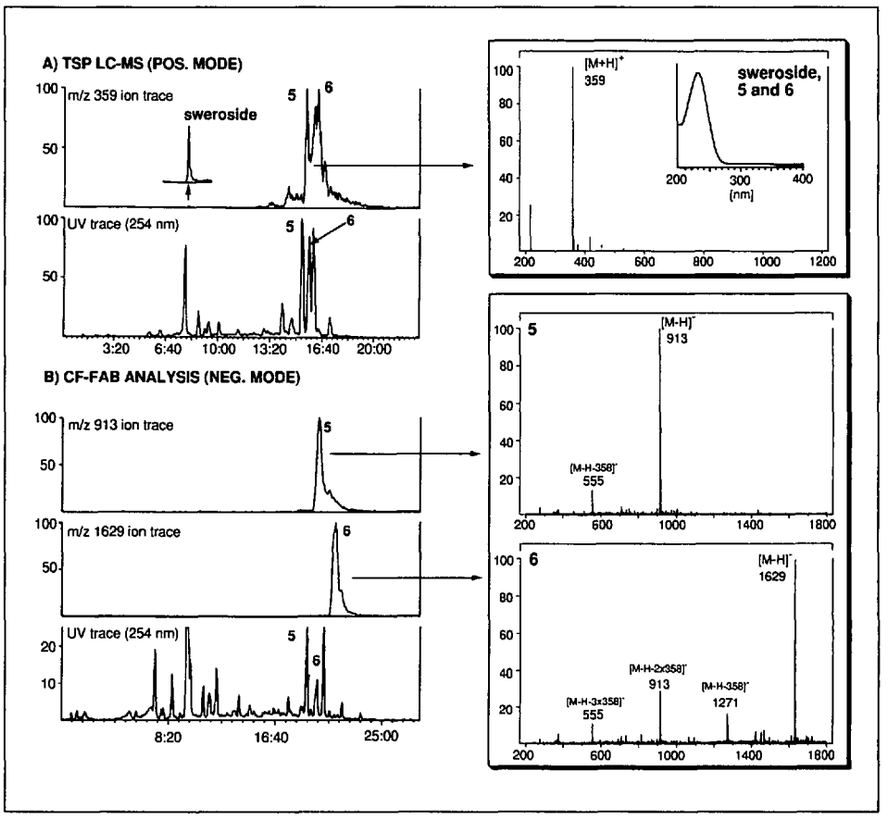


Fig. 2. Combined TSP (A) and CF-FAB (B) LC/MS of the enriched BuOH fraction of the methanolic extract of *Gentiana rhodantha* (Gentianaceae). HPLC: Column, RP-18 Novapak (4 µm, 150 × 3.9 mm i.d.); gradient, CH_3CN-H_2O (0.05% TFA) 5:95 → 50:50 in 30 min (0.9 ml/min). TSP (A): Positive ion mode; filament off; vaporiser, 90 °C; source, 230 °C; $AcONH_4$ 0.5 M (0.2 ml/min post-column). CF-FAB (B): Negative ion mode; FAB tip 50 °C; source 100 °C; glycerol 50% (v/v) (0.15 ml/min post-column); LC flow post column split 1:100.

HPLC coupled with nuclear magnetic resonance (LC/NMR), despite being known for over twenty years [15], is not yet a widely accepted technique, mainly because of its lack of sensitivity. However, the recent progress in pulse field gradients and solvent suppression, the improvement in probe technology and the construction of high field magnets have given a new impulse to this technique. LC/NMR has an important potential for on-line structure identification of natural products in plant extracts. Indeed, nuclear magnetic resonance (NMR) spectroscopy is by far the most powerful spectroscopic technique for obtaining detailed structural information about organic compounds in solution [16].

3.4. *Isolation of Active Principles and their Structure Determination*

Target compounds, either active in one bioassay or detected as potential new chemical entities, will then be isolated and their structure elucidated. This is achieved by fractionation using different preparative chromatographic techniques [17]. The fractions issuing from this first separation are submitted to biological testing and analysed by HPLC in order to localise the compound of interest. This procedure is repeated until the pure compound is obtained. Generally, several hundred grams of dried vegetable material are required for the isolation of several milligrams of the pure target compound. Once this has been achieved, the structure of the product has to be determined using methods such as UV and IR spectroscopy, NMR and MS. Interpretation of the spectra may lead directly to the structure of the isolated plant constituent. However, chemical reactions may be necessary to confirm a structure hypothesis deduced from spectroscopic data. This may require a relatively large amount of sample. These various steps may lead, in the best case, to the discovery of a new, previously undescribed, compound with interesting pharmacological properties which needs further investigation. In any case, if an efficient chemical screening procedure has been completed at the beginning, the chances of isolating an ubiquitous natural product should be very limited.

It must, however, be stated that when a new compound with interesting activities in several biological tests or pharmacological models has been discovered, one is still far from an effective therapeutic agent. This is only the beginning of a procedure, involving animal, clinical and toxicological testing, which may last years! At the present moment, out of 20 000 new molecules investigated by the pharmaceutical industry, only a single compound will reach the stage of commercialisation [18]!

4. Investigation of the Antifungal Constituents of *Bobgunnia madagascariensis*

There is presently a great deal of research underway to develop new antimycotics. This is because of the increased prevalence of systemic mycoses associated with AIDS infections. It is obvious that the fight against viral diseases such as AIDS or herpes is of high priority for numerous research laboratories. Plants are of great potential for their antiviral and antifungal properties. On the other hand, their antibacterial activity, although known for essential oils and in plants such as bearberry, is relatively

weak when compared with antibiotics of microbiological origin.

Bobgunnia madagascariensis (Leguminosae, formerly known as *Swartzia madagascariensis*) has been intensively studied at the Institute of Pharmacognosy and Phytochemistry in Lausanne because the root bark contains fascinating and extremely potent agents which are presently under clinical investigation. There are few really effective antifungal preparations currently indicated for the treatment of systemic mycoses and their efficacy is rather limited. Another area which is in need of new lead compounds is agrochemistry. Consequently, the investigation of higher plants for antifungal properties is of great importance at the moment. For the isolation of active compounds by activity-guided fractionation, bioautography is the method of choice. This technique combines TLC with a bioassay *in situ* and allows localisation of active constituents in a plant extract. Spore-producing fungi, such as *Aspergillus*, *Penicillium* and *Cladosporium* spp. can all be employed as target organisms in direct bioautographic procedures [19]. Since direct bioautography is not possible with yeasts such as *Candida albicans*, a simple and rapid agar overlay assay has been developed [20]. This contact bioautography technique relies on the transfer of active compounds from the stationary phase into the agar layer (which contains the microorganism) by a diffusion process.

More than 2000 plant extracts have been screened for their antifungal properties by the Institute of Pharmacognosy and Phytochemistry in Lausanne using bioautographic techniques. A dichloromethane extract of the root bark of *B. madagascariensis* gave extremely interesting results. Chemical screening data suggested the presence of compounds with original chemical structures in this extract. Phytochemical investigation of the extract led to the isolation of 14 new diterpene quinones. The major constituent, a phenanthrene-3,9-dione (**7**), was also the strongest inhibitor of fungal growth [21]. Its structure was elucidated by different chemical and spectroscopic techniques, with final confirmation by single crystal X-ray analysis. The absolute configuration was determined by X-ray analysis of the 4-bromobenzoate derivative. The antifungal activity of the major compound was compared with that of commercial antifungal agents towards a panel of commercial and clinical fungi, such as several *Candida* species and pathogenic fungi of the type *Aspergillus*. Activities considerably superior to those of amphotericin B and fluconazole were obtained against *Candida* species. This prompted the filing of a patent [22] and incorporation of seven of the isolated diterpene quinones in preclinical testing. *In vitro* assays determining the sensitivity of several fungal organisms to the compounds are underway. Cytotoxicity tests and first experiments on animals are also in progress.

The ultimate aim is to introduce new antifungal drugs which can be topically applied for dermatomycoses, or as oral formulation for the treatment of systemic mycoses associated with HIV infections.

5. Conclusion

Even though only a limited selection of the therapeutic applications and bioactivities of plant products is outlined here, a general idea is given of their extraordinary usefulness. Not only do natural products themselves possess significant pharmacological properties but they also provide a source of very important lead compounds which can be modified for the development of new therapeutics. The introduction of new bioassays and the expansion of screening programmes will provide a valuable source of new drugs in the future, a reservoir which should be sensibly exploited before the large-scale destruction of these natural resources goes too far. The most effective strategy is to perform multidisciplinary work on the development of drugs from plants, a task that can only be effectively tackled by collaboration between botanists, ethnobotanists, pharmacognosists, phytochemists, biologists, pharmacologists and medical doctors.

Acknowledgements

The Swiss National Science Foundation (FNRS) is gratefully acknowledged for financial support.

Received: September 6, 2000

[1] O. Potterat, K. Hostettmann, in 'Encyclopedia of Environmental Biology, Vol. 3', Academic Press, London, **1995**, p. 139.
[2] M. Hamburger, A. Marston, K. Hostettmann, in 'Advances in Drug Research, Vol. 20', Ed. B. Testa, Academic Press, London, **1991**, p. 167.
[3] G.M. Cragg, D.J. Newmann, K.M. Snader, *J. Nat. Prod.* **1997**, *60*, 52.
[4] G.A. Cordell, *Chem. & Ind.* **1993**, *Nov.*, 841.
[5] H.J. Woerdenbag, N. Pras, W. van Uden, T.E. Wallaart, A.C. Beekman, C.B. Lugt, *Pharm. World Sci.* **1994**, *16*, 169.
[6] M. van Vugt, A. Brockman, B. Gemperli, C. Luxemburger, I. Gathmann, C. Royce, T. Slight, S. Looareesuwan, N.J. White, F. Nosten, *Antimicrob. Agents Chemother.* **1998**, *42*, 135.
[7] A.L. Miller, 'St. John's Wort (*Hypericum perforatum*): clinical effects on depression and other conditions', *Alternative Medicine Rev.* **1998**, *3*, 18–26.
[8] K. Hostettmann, M. Hostettmann, 'Xanthones', in 'Methods in Plant Biochemistry', Ed. J.B. Harborne, Academic Press, London, **1989**, p. 493–508.
[9] K. Ingkaninan, C.M. de Best, R. van der Heijden, A.J. Hofte, B. Karabatak, H. Irth, U.R. Tjaden, J. van der Greef, R. Verpoorte, *J. Chromatogr. A* **2000**, *872*, 61.
[10] K. Hostettmann, J.-L. Wolfender, S. Rodriguez, *Planta Med.* **1997**, *65*, 64.
[11] K. Hostettmann, B. Domon, D. Schaufelberger, M. Hostettmann, *J. Chromatogr.* **1984**, *283*, 137.
[12] J.-L. Wolfender, K. Hostettmann, in 'Phytochemistry of Medicinal Plants', Ed. J.T. Arnason, R. Mata, J. Romeo, Plenum Press, New York, **1995**, p. 189.
[13] J.-L. Wolfender, S. Rodriguez, K. Hostettmann, W. Wagner-Redeker, *J. Mass Spectrom. and Rapid. Commun. Mass Spectrom.* **1995**, S35.
[14] W.G. Ma, N. Fuzzati, J.-L. Wolfender, C.R. Yang, K. Hostettmann, *Helv. Chim. Acta* **1994**, *77*, 1660.
[15] N.Watanabe, E. Niki, S. Shimizu, *Jeol News* **1979**, *15 A*, 2.
[16] K. Albert, *J. Chromatogr. A* **1995**, *703*, 123.
[17] K. Hostettmann, A. Marston, M. Hostettmann, 'Preparative Chromatography Techniques: Applications in Natural Product Isolation', Springer, Berlin, **1998**.
[18] K. Hostettmann, 'Tout savoir sur le pouvoir des plantes, sources de médicaments', Favre SA, Lausanne, **1997**.
[19] A.L. Homans, A. Fuchs, *J. Chromatogr.* **1970**, *51*, 327.
[20] L. Rahalison, M. Hamburger, K. Hostettmann, M. Monod, E. Frenk, *Phytochem. Anal.* **1991**, *2*, 199.
[21] F. Schaller, L. Rahalison, N. Islam, O. Potterat, K. Hostettmann, H. Stoeckli-Evans, S. Mavi, *Helv. Chim. Acta* **2000**, *83*, 407.
[22] K. Hostettmann, F. Schaller, Antimicrobial diterpenes, United States Patent No 5,929,124, **2000**.