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Review

Biodiversity and drug discovery — a symbiotic relationship

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Abstract

The profound developments in natural products drug discovery in the past few years are discussed, and the importance of a global approach to biodiversity and drug discovery involving natural products for the early part of the 21st century is presented.

Keywords: Biodiversity; Drug discovery; Intellectual property rights; Future opportunities

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1. Natural products in drug discovery

Several previous articles have discussed various aspects of the importance of natural products in drug discovery and how they may have a critical and distinctive role to play in the future (Farnsworth et al., 1985; Soejarto and Farnsworth, 1989; Cordell, 1990, 1993a, 1995a; O'Neill and Lewis, 1993; Nisbet and Moore, 1997; Shu, 1998). The changing strategies for natural product chemistry in the past few years have also been reviewed (Cordell, 1995b); although these strategies are changing so rapidly that already this article is outmoded. This present article will attempt to link these developments with some global issues, and begin to present a convergent vision of many disparate views of the development of medicinal and biological agents from natural sources. Elsewhere, I have described these concepts as the "yin" and "yang" of natural products chemistry (Cordell, 1998, 2000). This article is, in part, a commentary on finding a middle way, an as yet untrodden path in drug discovery, for the global health benefits of humankind.

At a workshop I attended a few years ago at the Esalen Institute on Big Sur, the humanist–philosopher Brother David Steindl-Rast spoke eloquently of the need to be grateful in all parts of our lives. Indeed, in his book *Gratefulness, the Heart of Prayer* he extends this notion to the continuous acknowledgment and appreciation of our surroundings, how they came to be there, and how they are a part of our everyday lives (Steindl-Rast, 1984).

If we consider this concept in terms of Gaia, Mother Earth, as propounded by James Lovelock, we see that we have much for which, on a moment to moment basis, we should be grateful. Lovelock speaks of a living Earth, the continuous interdependence, interplay, and recycling of all biological material, especially including ourselves, of the constant reverberation which results from a single perturbation to the delicate balance that is the living organism Earth, and of the need to recognize, and become reconciled with, our transitory presence (Lovelock, 1982).

These two ideas, gratefulness and constant transition, intertwine with the concepts which are discussed here as we consider the impact that natural products will have in the discovery of medicinal and biological agents in the next 50 or so years. Perhaps we can begin our pathway of gratefulness by acknowledging that natural products have provided, and continue to provide, essential materials for shelter, for furniture, for food, for clothing, for writing, for coloring materials, for weapons, for gifts, and for the treatment of numerous diseases (Balick and Cox, 1996). The value of these plant products is estimated to be in the region of $500–800 bn. annually.

Sometimes, the medicinal "gifts" from nature have been codified, and the names of Dioscorides, Galen, and Gerard are well-known (Balick and Cox, 1996). The countless anonymous authors who compiled the treatises of the Chinese, Ayurvedic and other systems of traditional medicine, as well as the untold scribes and knowledgeable shamans who passed on their locally valued information in a more personal manner, should also be gratefully acknowledged (Balick and Cox, 1996).

When, in about 1524, Paracelsus wrote in his "Archidoxa" of the "Arcanum" he was referring to the need to discover the essential active component, the "secret", of a treatment, whether it was animal, mineral or vegetable (Pachter, 1951; Di Stefano, 1994). Not knowing that compounds, as they would later be called, even existed, he encouraged the search for such species in natural medicaments. "It does not matter that rhubarb is a purgative. The question is: What purges? ... Names do not have virtues. Substances do" (Pachter, 1951; Di Stefano, 1994).

This search for virtuous substances began in the 1780s with the work of Scheele on the organic acids of plants (Sneader, 1985). Bioactive principles were sought in earnest in the very early part of the nineteenth century, when, in a period of fifteen or so years, the investigation of several renowned medicinal plants led to the discovery of a number of the most significant biologically active alkaloids (Cordell, 1981; Sneader, 1985). Some of these alkaloids, morphine, atropine, papaverine and codeine, subsequently became the cornerstones of many aspects of drug discovery today (Sneader, 1985; Foye et al., 1995).

Farnsworth and colleagues indicated (Farnsworth et al., 1985) that globally there were 119 compounds from 90 plants which were used as single entity medicinal agents. Significantly, 77% of these were obtained as a result of examining the plant based on an ethnomedical use, *and* are employed in a manner that approximates that use. The number of plants used as medicinal agents in commerce globally is unknown, but is at least 1000 in China alone (Duke and Ayensu, 1985). There is in both medicine and pharmacy, not to mention in the lay public, a serious lack of acknowledgment and appreciation that such compounds continue to come from natural sources. Even when a physician prescribes an antibiotic, there is little awareness that a fungus or a bacterium, probably associated with some decaying plant material, was the original source of that compound.

Modifications to natural products, with a view to enhancing activity or selectivity and reducing side effects or toxicity, developed as organic chemistry grew in the late 19th century (Sneader, 1985). Aspirin was one of the earliest of these chemically modified natural products and recently celebrated 100 years as a commercial entity (Reisch, 1997). Such modifications to natural products are rarely so simple today, (one exception may be taxol and taxotere), and frequently the relationship to a natural product may be barely discernible. However,

as O'Neill and Lewis have pointed out, half of the best-selling pharmaceuticals in 1991 were actually based on a natural product precursor or pharmacophore (O'Neill and Lewis, 1993).

The lack of appreciation of natural products as an important source of approved drugs in the United States was illuminated more recently by Cragg and associates. They conducted an analysis of drugs approved by the Food and Drug Administration in the United States in a 12-year period (1983–1994) and found that 157 of 520 drugs (30%) approved were natural products or their derivatives (Cragg et al., 1997a). When focused efforts are made to discover natural products for clinical use, the success level rises dramatically. Thus, in the same period, 61% of anticancer agents approved were natural products or their derivatives. In the absence of targeted programs involving natural products, there was no success; thus, there were no analgesics, antidepressants, antifungals, antihistamines, antivirals, anxiolytics, or cardiotonics derived from natural products which were approved in this time period (Cragg et al., 1997a).

2. The discovery process

In order to understand the contribution that natural products can, will, and indeed must, make to the discovery and provision of medicinal and biological agents in the future, it is necessary to establish what are the steps in this discovery pathway and what are the sources of such agents. Some of the characteristics which would enhance the role of natural products in this process are then discussed.

Succinctly, the initial steps in the drug discovery process necessitate that many potential entities are evaluated against a biological assay or panel of assays (a screen), and those which achieve a certain criterion for activity are moved to the next phase of evaluation (Kuhlmann, 1997). It is the strategies which are currently being employed for these processes which will define the successful pharmaceutical company 15–20 years from now.

For most of the major pharmaceutical companies, drug discovery is seen as potentiating the number of chemical entities which are evaluated against a particular biological screen in the minimum time. The collection of "compounds" to be evaluated against each screen as it comes on line is termed a "library". Companies spend enormous amounts of time, money and effort deciding how to enhance or optimize these libraries for particular screens (O'Neill and Lewis, 1993). Actually, these libraries could also be regarded as "bank deposits" because they are truly a core future asset of the company, and, particularly in the case of natural products and their extracts, may well increase substantially in value as the biodiversity continues to decline (see later). The National Cancer Institute has developed its own program and repository of organism collections from the plant and marine areas (Cragg and Boyd, 1996).

The acquisition of chemical entities which can be added to the library is an interesting and challenging process. There are relatively few sources of compounds for evaluation: natural, synthetic, semi-synthetic, genetic engineering, and combinatorial chemistry. From a discovery perspective, the key concept in developing a library is to maximize the chemical diversity, or more critically to maximize the pharmacophoric diversity, using these potential sources creatively.

The evaluation of the library of samples typically involves a receptor-, enzyme- or cell-based assay which has been optimized for automation. Samples are contained in 96- or 384-well plates, and all aspects of the process are usually fully automated. Further details will be described subsequently. After the sample library has been screened, automated data analysis provides a prioritized list of samples for further consideration as an "active". Subsequent chemical and biological experimentation is aimed at sorting and prioritizing these entities further until "leads" are identified which can be advanced to more extensive biological and formulation studies (Kuhlmann, 1997).

A pharmaceutical company typically supports drug discovery in a number of therapeutic areas to which it is committed for product development. Consideration of the therapeutic target areas is made at the highest corporate levels, and typically involves market economists, clinicians, etc., Scientists experienced in the fundamental aspects of the discovery process are not usually involved. The diseases which are chosen for the development of new therapeutic agents are frequently those for which the company has biological, pharmaceutical and clinical expertise, and the market is already established or is projected to be very large (Thayer, 1998a). This is clearly, in part, due to the extraordinary costs ($500–600 million) of bringing a new drug to the marketplace and the consequent need to recover those costs within the lifetime of the patented material (Kuhlmann, 1997). Thus, some of the diseases which are common therapeutic targets are cancer, heart disease, lung diseases, pain and inflammation, anti-infective agents, anti-HIV agents, diseases of aging, and diabetes.

While some of these diseases are also important globally, other diseases, including malaria, schistosomiasis, filariasis, diarrhea, hepatitis C, and intestinal parasites, are responsible for substantially more deaths worldwide on an annual basis. Diarrhea is, for example, responsible for about 5 million deaths in infants (0–4 years) annually. Regrettably, relatively little drug discovery in these areas is being conducted by the major pharmaceutical corporations at the present time (Cragg et al.,

1997a), although a new WHO initiative for malaria may serve as a model for other global diseases.

No area of drug discovery has changed more significantly in the past 10 years, as a result of biological and technological innovation, than the rate at which primary screening is being conducted (Goldman, 1995). In the industrial setting of a major pharmaceutical company, the activities of four groups blend together for the purposes of primary screening. One is a group devoted to sample generation and acquisition, another is responsible for the development of high throughput and secondary bioassays, and a third is responsible for the technological aspects of automation. Finally, there is the group which is responsible for the data collection and analysis (Babiak, 1997).

Sample generation for the library to be screened may take several different forms, including, buying samples from commercial chemical catalogues, from academic laboratories, or small companies dedicated to providing samples. In-house synthetic chemistry programs typically provide a large array of compounds, albeit of quite limited structural diversity based on previous discovery programs. Many companies are also generating samples for primary screening through various combinatorial chemistry approaches (Gordon et al., 1994; Myers, 1996), which are under constant review (Ecker and Crooke, 1995; Myers, 1996). Finally, there are the natural product samples, which might be purified compounds, or crude or semi-purified extracts of plants, microbes (fungi, bacteria) and animals from terrestrial or marine sources. Each of these areas requires specialized acquisition and sample handling and processing. These samples are usually stored in 96-well plates (''master'' plates) which can then be accessed automatically to generate the plates of samples for a particular bioassay (Goldman, 1995).

With the possible exception of antidiabetic drug discovery, animals, or even animal organ preparations, are now rarely used in primary screening (O'Neill and Lewis, 1993). All of the initial biological evaluation for therapeutic purposes is conducted with cell, enzyme or receptor-based assays, although a variety of insects and marine and terrestrial invertebrates are used for other aspects of bioactive compound discovery. Substantial innovation has been exercised in developing genetically engineered assays, and in developing new ways to indicate, often qualitatively, a biological response (Sweetnam et al., 1993). For a given therapeutic area there may be 5–10 automated bioassays generated over a period of time in order to evaluate the available library of samples. There are substantial issues regarding many of these primary assays and how they have evolved. One of these is the need to have assays which are fully automated, targeted towards new enzyme and receptor targets, and able to offer an unequivocal response for a particular sample. This has led to significant compromises with respect of the relevance with respect to a therapeutic end-point. In addition, the biological novelty of the assay may mean that there is no positive control compound available with which to compare any ''hits''. Thus the value of the ''hits'' may only become apparent through secondary or tertiary bioassays.

Automated sample preparation, automated assay preparation and automated data collection are conducted by a single robot capable, once programmed, of operating 24 h a day. One million or more assays per robot may be conducted per year as a minimum (Goldman, 1995; Kuhlmann, 1997); and for many companies the number is significantly higher. This, in spite of the ''downtime'' that is inevitable between assay runs which allows for reprogramming of the robot such that the next assay can be run. Given the size of a companies' library of samples to be screened, the assays used for primary screening may be operated for only 1–2 months before other assays are brought on-line.

In academia, similar strategies, albeit greatly reduced in scope and scale, are being used in drug discovery programs. Our National Cooperative Drug Discovery grant program for plant-derived anticancer agents has been described previously on several occasions (Cordell et al., 1991; Cordell, 1993b; Cordell et al., 1993, 1994; Kinghorn et al., 1995), and only brief details will be mentioned here. We evaluate extracts from approximately 500 plant samples per year in several cell-based (usually 5–8 cell lines) semi-automated assays. Approximately 1000 assays per month are run. Although some cell lines have been changed in the past 3–5 years, for consistency and comparison, certain cell lines have been maintained for many years as a part of the primary screening effort. When new cell lines are introduced, either the complete, or a modified library, of extracts is evaluated against the new system. Data collection and analysis allows the generation of the complete biological profile of an extract after data is received from bioassays that are being conducted on the same samples at Research Triangle Institute and Bristol Myers Squibb, who are our partners in the program. This approach is suitable for many types of drug discovery program based on plants in an academic or small research institute setting. In addition, it will find application in the future for the chemical and biological standardization of phytotherapeuticals.

The advances which have occurred in the structure elucidation of natural products during the past decade or so have been well-discussed elsewhere (Martin and Zetzker, 1988; Cordell, 1995b). Advances in probe design have allowed for smaller samples to be analyzed for both proton and carbon spectra, and advances in software control systems have permitted dramatic improvements in the range of spectra that can be obtained. Many kinds of two-dimensional spectra are now available at increasingly high field (500, 600 and

750 MHz) to augment the information from the one-domain spectra, and these routine spectra can typically yield substantial information regarding short- and long-range proton–proton correlations and proton–carbon correlations, and proton–proton distances (Cordell, 1995b). In most cases, it is now possible to derive unambiguous proton and carbon assignments for almost all classes of isolated natural products using these techniques.

Structure modification of active metabolites is required in order to explore the preliminary information regarding the functional groups in the molecule needed for activity (Foye et al., 1995; Michne, 1996; Kuhlmann, 1997). This may be followed by combinatorial chemistry around either the whole molecule, if that is necessary for activity, or with that portion of the molecule which is essential for activity, the so-called pharmacophoric unit (Ecker and Crooke, 1995; Michne, 1996). Depending on the bioassay, and knowledge of the enzyme interaction, it may be possible to use computer-aided design to seek alternative groups or conformations of the molecule which might permit an improved enzyme inhibition at the active site (Salemme et al., 1997).

Information management can have a major impact in two separate areas of the discovery process. For dedicated natural product programs, and those programs which see value in natural products other than as constituents in a chemical library, prior knowledge about the indigenous use of the plants, of the known biological activities of the various plant extracts, and an awareness of the compounds which have been isolated from them, is critical (Cordell et al., 1991). Either such information can be used initially as a directive for the collection program, or at the stage when the samples to be presented for primary screening are being formulated. In addition, another important opportunity is at the stage of prioritization for fractionation after "actives" have been determined. In our programs, we routinely use such information in both circumstances. We have discussed the use of prior information in the conduct of a collection program for the discovery of fertility regulating agents derived from plants (Fong et al., 1990). In this instance a list of over 5000 plants used ethnomedically for fertility regulation was processed according to desired criteria to provide a list of 400 plants for collection on a global basis. We have performed similar analyses for cancer, diabetes and malaria, and for antimycobacterial activity, also using the NAPRALERT database (Farnsworth et al., 1995).

In our HPLC/ESMS/bioassay-based dereplication program (Cordell et al., 1997; Shin et al., 1999) we use the NAPRALERT database to correlate information on the mass of the compound(s) in the area of biological activity with those compounds that are known of that mass, whether isolated previously from the genus or not, and those compounds of that mass which are known to have that biological activity. This enables the direct prioritization of the extracts for fractionation on the basis of a probability to yield a known or new biologically active metabolite.

Recently, another technique using LC/^{1}H NMR has been used to help in the identification of compounds present in active extracts (Wolfender et al., 1997; Cavin et al., 1998). This technique, although very powerful in the stop-flow mode, at present lacks sensitivity in the continuous flow mode, is of limited use if the compounds are new, and uses solvent systems that are not conventional for NMR. Therefore, the possibility of unpredictable solvent shifts must be considered. It also suffers from a direct lack of correlation with the biological assay. Future developments though will undoubtedly address some of these issues for this important technique. The use of selective TOCSY experiments was recently described to obtain proton data for the analysis of a simplified mixture of synthetic compounds (Sharman, 1999). It will be interesting to see if this technique can be applied to selected natural product mixtures for the analysis and quantitation of the active principles or of impurities.

There is substantial confusion in the literature with respect to the terms "hit" and "lead" as applied to either purified compounds, synthetic sample mixtures, or natural product mixtures. At the end of the primary screening process, a number of samples will show potentially interesting activity, either through an arbitrary cut-off, or through comparison with a known biological marker. These actives (extracts or compounds) are sometimes referred to as "hits". However, more factors are usually involved, and, even prior to consideration for further development, other assays or details are frequently necessary to prioritize the list of active samples (Kuhlmann, 1997).

A "hit" is better referred to as a compound which is active, where the structure is established, where there is possibly some novelty (it may already be patented), and most importantly in many respects, is available. The latter is important because a true "hit" will be needed in reasonable quantity for additional chemical and biological studies. It is from these further studies on a series of compounds that are regarded as "hits" that compounds of even higher priority, a "lead", may evolve (Michne, 1996). A "lead" is a compound which has well-defined purity, possesses genuine structure–activity relationships for the target assay(s), has a well-defined minimum structure for activity, has selective activity, and is potent. The latter is a somewhat controversial qualification for candidacy for a "lead", and its value as a criterion may depend on several factors including availability, intended pharmaceutical use, and comparison with other known clinically useful products. Depending on the therapeutic target, in vivo activity may also be established, together with the margin of

safety. Such an entity will be patented together with a number of close relatives.

The overall process of taking a compound from the stage of a "lead" through the successive levels of evaluation to a marketable product have been described on a number of occasions (Sneader, 1985; Kuhlmann, 1997), and much of the work is dominated by the requirements for evaluation developed by the US Food and Drug Administration (Vogt and Montagne, 1984; Young, 1995). Typically, lead development involves a series of pharmacology and toxicity studies in several species of animal, bioavailability and pharmacokinetic studies, and formulation studies depending on the proposed route(s) of administration. Following the filing of an application for an investigational new drug (IND), trials in humans then follow, and are categorized as Phase I, Phase II and Phase III clinical studies. These studies typically take 4–7 years to complete depending on the disease state. At this point, application for approval as a new drug entity (NDA) is sought from the FDA. If this is approved and the drug is marketed, post-marketing surveillance (Phase IV) studies are required examining efficacy and side-effects on a long-term basis (Kuhlmann, 1997).

3. Intellectual property rights

One of the most contentious areas in natural products chemistry and biology at this time is that of intellectual property rights. There are many aspects to this broad topic, most of which are beyond the breadth of this discussion; some highlights are worthy of mention, however (Reid et al., 1993). Long before the Convention on Biological Diversity, the so-called Earth Summit, it was recognized that countries had the right, within their legal boundaries, of ownership of their biological property, both marine and terrestrial, and that indigenous peoples also had the right to protect and seek compensation for the knowledge which they had developed, over the generations, based on their local biodiversity (Reid et al., 1993; Cragg et al., 1997b). Many scientific societies and groups developed policies and statements which reflected concerns in these areas (Cordell, 1993a).

The Convention on Biological Diversity (CBD) codified many of these intellectual property concerns and also instructed nations to develop plans to catalog and preserve their biodiversity and their indigenous knowledge (Anonymous, 1992a; Cragg et al., 1997b). The CBD has now been ratified by 178 countries with notable non-signatories being the United States and Thailand. The Convention asks the Contracting Parties to "endeavor to create conditions to facilitate access to genetic resources for environmentally sound uses...". Some countries have chosen to introduce strict regulations to control access to their biome (Anonymous, 1995), others have, for the most part, chosen to encourage interested parties to work with local personnel, and to develop prior relationships which will foster constructive development without exploitation. In our experience at UIC we have worked diligently with a number of countries and local groups in the past ten years to develop a series of agreements for access to the biome in exchange for a number of different compensatory packages. In some instances however, we have now chosen not to continue to collect plant samples in certain countries, either because of unacceptable and restrictive policies, because the penalties in the event of not meeting requirements were so onerous as to be restrictive of our academic rights, or because our industrial partners indicated that they would not biologically evaluate extracts from these countries. As stated elsewhere (Cordell, 1995b) "the Earth Summit...may, in the long term, be one of the most profound steps ever taken in natural products chemistry". However, without a greater and deeper understanding of the diverse issues, the outcomes for all parties may not necessarily be positive.

Protecting and compensating local groups for their indigenous knowledge and for providing access to the biome is a reasonable expectation for both those who hold the resources and those who are seeking them (Reid et al., 1993). Compensation may take any one of a number of forms, and we have been involved in many of these initiatives, including developing training programs for individual personnel on-site and at UIC, developing laboratories through the provision of equipment, and providing symposium programs. Longer term forms of compensation may include sharing in any royalties or productivity payments and offering first right of refusal for indigenous crop development (Reid et al., 1993). This area is evolving rapidly and a new approach developed by UIC will be described subsequently.

Another route for the acquisition of biologically significant compounds which pharmaceutical companies use is to license compounds from third parties (academia, research institutes, or other companies). These agreements usually involve up-front cash payments or exchange of stock, followed by milestone payments depending on the performance of the compound, or its derivatives, in the process towards a finished, marketable product. Thus, it is essential that academic institutions have very strong technology transfer groups to negotiate these relationships (Thayer, 1992).

For many years a myth existed that natural products could not be patented. A glance at almost any issue of *Chemical Abstracts* verifies that this is not the case. The situation is that patenting the compound, new or old, must be tied to a non-obvious biological activity. Many years ago, for example, we patented a very simple, natural quinone which had (non-obvious) in vivo anticancer activity (Ogura et al., 1978). For licensing

purposes, of course, the acquiring company typically wishes for that level of exclusivity when such an asset is purchased. Higher value for a patent is placed on a compound with structural novelty and with some derivatives produced which offer information regarding structure activity relationships. An even higher premium is engendered if the compound is novel, there are some structure activity relationships established, and the biological mechanism of action is unique. On the other hand, there are also individual natural products which are yielding over a billion dollars annually in sales which were never patented!

It should also be mentioned that there is a direct conflict between the CBD of 1992 and the World Trade Organization (WTO) agreement of 1995 with respect to intellectual property ownership. The former endorses the concept of governments or local groups within nations holding the intellectual property rights to their indigenous species and the molecular entities derived from them. The WTO agreement on the other hand suggests that ownership of the intellectual property rights lies with the inventor of the technology, resulting in a profit-based system of rewards. Thus, for natural products there is a tension to be resolved and negotiated as an aspect of the prior permission to afford access. In addition, as a result of this tension, a number of curious scenarios have developed in the past few years (Pollack, 1999). For example, some institutions have patented preparations of well-established traditional medicines or ethnopharmacological preparations, such as neem, turmeric and ayahuasca. The recent cancellation of a turmeric patent in this area, based on an appeal (Pollack, 1999), and the request for re-examination of the ayahuasca patent by indigenous groups (Dean, 2000) are probably appropriate developments. Yet there is also an attempt underway by a Japanese company to patent various curry preparations (Makoto and Sachiyo, 1999).

For the future there will need to be a series of new criteria propounded for natural products to be successful in this milieu. On one side indigenous groups must feel that their rights are not being stolen from them, and those making substantial investment in the development of products must feel that they can protect their added value. Sharing of resources and knowledge therefore becomes critical. In addition, natural products must find a new discovery niche, where novel biology would play a far more dominant role in prioritization for protection than novel chemistry. The reason for this is that given the number of known natural products (vide infra), and the intensive innovation underway in developing new bioassays, there is a very high chance that many of the "old" compounds will be found to have new, interesting and potentially exploitable biological activities. Unless these discoveries are encouraged to be protected, many extremely interesting avenues for the development of new medicinal agents based on natural products will be lost. We will need to look far more critically at potential therapeutic benefit, and not primarily at chemical uniqueness. The latter attribute can be addressed during a biological potentiation stage. As an example, we can cite the recent development (Pisha et al., 1995), and subsequent patenting (Das Gupta and Pezzuto, 1997), of betulinic acid, which exhibits specific cytotoxicity against melanoma cells, and also possesses in vivo antiproliferative and cancer chemopreventive activities.

4. Differing philosophies of the role of natural products

The classical view of what constitutes a drug in the United States, and much of the developed world, is a single chemical entity which, in its purest form, has been rigorously evaluated for its biological, pharmacological, toxicological, and clinical effects (Kuhlmann, 1997). When the product is synthetic and has stereogenic centers, then the focus is also on the chiral purity of the biologically active form (Stinson, 1997; McCoy, 1999). The market for these agents is approaching $100 billion per year in a range of drug categories (Stinson, 1999).

Bioactivity-directed fractionation is the process whereby the compound responsible for a given activity in a natural product extract is isolated and characterized (Cordell et al., 1997). As the compound proceeds through the various stages of the development process, so it will be modified through semi-synthesis with a view to enhancing potency, reducing toxicity or modifying solubility. If the biologically active area within the molecule, the pharmacophore, can be identified, synthetic medicinal chemistry around this unit may be initiated in order to achieve the same purposes (Sneader, 1985; Michne, 1996). Only in extremely rare instances, does the isolated natural product itself serve as a "magic bullet".

A second type of natural product preparation is the multicomponent compound mixture. Such a preparation, which may be comprised of several closely related, biologically active compounds (e.g. gingko flavonoids, capsaicinoids, valepotriates), is frequently marketed when separation of the individual chemical entities is very difficult, or not cost-effective, and/or not required by regulation. Mixtures of this type are usually characterized chemically or, rarely, biologically. Some efforts are underway in the United States to develop analytical techniques for the standardization of phytotherapeuticals (Schutt, 1998; Srinivasan and Kucera, 1998). However, they fail to consider even minimum standards for both chemistry and biology which must be required in the future.

Another type of natural product entity is the single plant extract. Frequently, this is either a lyophilized

aqueous extract or an alcoholic extract which has been dried and powdered. Expressed oils and essential oils would also be included in this category. These are very complex mixtures, and standardization chemically (other than a gross chromatographic profile) is impossible, although analysis is important for the purposes of authentication and assuring the absence of adulteration (Wagner et al., 1984). Biological standardization is to be desired in these cases. Also marketed are numerous multiple plant extracts, for which any assay other than biological standardization is extremely difficult.

Single plant materials, in their dried and powdered form are utilized in many parts of the world as precursors to teas, or for the preparation of poultices. Many are also found in tablet or capsule form. Multiple plant mixtures are also marketed in this manner in many countries throughout the world. Macroscopic examination and extraction and chromatographic analysis may provide some evaluative information, but again, a biological assay to assure an acceptable level of activity is essential. At the local level, fresh plant material is often regarded as being crucial for the preparation of teas, etc., in order to optimize effectiveness (Balick and Cox, 1996). Thus we must recognize and appreciate that different countries will have quite different considerations regarding the regulation of their various drugs. In all instances, analysis for heavy metals and a range of common herbicide and pesticide residues is necessary. When this is done, unacceptable pesticide contamination is by far the most common reason for rejection of material for human consumption. Thus, these levels must be controlled very strictly.

Another important opportunity for natural products which has emerged in recent years is their role as nutraceuticals. These are compounds with a health beneficient or aging prophylaxis effect which can be added to common foodstuffs.

With the wide variety of synthetic chemicals which can be made available for a given screen, especially if combinatorial chemistry is used in the initial stage of the discovery process (see earlier), the very serious question arises of ''Why include natural products in drug discovery?'' There appears to be an underlying assumption in many biodiversity-rich, less-developed parts of the world, particularly as expectations concerning compensation for local plant materials and indigenous knowledge have risen, that natural products are an integral aspect of all drug discovery programs in the pharmaceutical industry. Nothing could be further from the truth. Several major pharmaceutical companies in recent years have totally eliminated their efforts involving natural products, both in discovery and in development. Other companies scaled down these efforts as combinatorial chemistry became the ''chic technic''. In order to understand this phenomenon, we need to take a closer look at the changing pace of the discovery process, and examine what it is that natural products can offer to the process.

Earlier, some of the strategies being used by industry for the initial phases of the discovery process were described. These strategies are based on balancing chemistry and biology. For example, it is estimated (Kuhlmann, 1997) that it may require the evaluation of 50,000–100,000 compounds in order to obtain a single marketable drug. Not all ''leads'' will yield a drug, nearly all (49 of 50) of the compounds which show promise at an early stage in the development process will fail when put into more advanced animal models. Some compounds will produce unwanted side effects in humans, or simply may not work, or may not offer improvements over existing products. Hence the (perceived) need to evaluate large numbers of samples against each screen. As discussed previously, companies are now typically working in the range of screening at least a million samples per year using a single robot (more if combinatorial chemistry is used) (Kuhlmann, 1997). Such a robot may conduct five or six different assays in the course of a year. On the other hand, it is estimated that a chemist can synthesize 200–300 compounds per year, or possibly isolate and characterize 100–150 natural products per year. Thus the numbers (personnel, time, compound accrual rate) indicate the need for external acquisition programs and extensive compound libraries.

For the purposes of this discussion however, the question is ''What can natural products bring to the bioassays?''. To answer this question let's consider the chemical library (bank vault) of a medium size pharmaceutical company. They probably have plated out into 96-well storage plates about 250,000 synthetic compounds, approximately 25,000 extracts of microbial organism fermentations, possibly 8000 plant and marine extracts (representing organic and aqueous fractions), and about 1500 purified natural products derived from plant, marine, and microbial sources. Thus natural products from marine and plant sources may be only about 3% of the whole library. At that level of participation can they be expected to effectively contribute? How can this paradigm be changed to reflect natural biodiversity? It is worth mentioning at this point that NAPRALERT lists over 135,330 isolated and characterized natural products derived from plants with 5750 different skeleta (Quinn-Beattie, 1999).

5. Issues and opportunities for natural products

Even though there is an awareness of the history of natural products in relation to current medicinal agents, and it is well recognized that natural products offer a diversity of structure which simply cannot be matched through even the most active imaginations of the synthetic organic chemists, questions remain.

Why has pharmaceutical industry rethought its involvement with natural products? What are some of the issues which have made natural products less competitive than synthetic products in the follow-up stages to high throughput screening? As a result, what are the opportunities for natural product chemists?

When complex extract matrices derived from plant, marine, or fungal sources display activity in a biological screen there is a need to isolate and characterize the active principle(s). This deconvolution process can be both expensive and time-consuming. Corley and Durley have estimated that for a plant extract this may take $50,000 and 3 months of work (Corley and Durley, 1994); assuming that the biological screen to be used for the purposes of activity-guided fractionation is still functionally available.

If the active principle can be isolated from the available sample, and interest continues in the compound, then a recollection of the plant or marine organism, or a refermentation of the microorganism may be needed. Sometimes, the originally observed biological activity is not reproducible on recollection. Although this happens less frequently with the refermentation of fungi and bacteria, in our experience it may happen up to 40% of the time with the recollection of plant materials. The now classic instance of the recollection of *Calophyllum lanigerum* var. *austrocoriaceum* for an anti-AIDS drug discovery program has been well documented (Dawes, 1992). Clearly, this is a very serious issue to be addressed.

Even if the active compound can be obtained from the large scale recollection of a plant or a marine organism, the yield may not be adequate to supply material for chemical modification in order to investigate the structure activity relationships, enhance potency, etc., prior to a decision with respect to development.

Biologically active natural products from plant, fungal and marine sources are often very complex in structure and possess many chiral centers. Such complications may place these compounds in a situation where they are refractory to synthesis, or at least difficult to produce in a timely manner, and in adequate quantity for additional studies.

For these, as well as some other reasons not discussed here, the fact is that **pharmaceutical companies do not need natural products as an essential component of their drug discovery programs**. This may come as a surprise to some natural product chemists and biologists, particularly those working in less-developed countries.

The issues raised above are not easily resolved for plant and marine-derived samples. Thus, in order for the biological (chemical) diversity of natural products to participate in drug discovery programs other opportunities which natural products might offer to the screening process must be considered. For that to be achieved, it is important to ask and answer some critical questions: What is the role of biodiversity in presenting chemodiversity to the biological screen? How can natural products be made more amenable to screening? How can the chemical diversity of natural products be enhanced? Are we fully utilizing all of the available chemical and biological information from plants and marine organisms that has accrued over the millennia in the discovery process? If natural products are to continue to be a part of the initial drug discovery process, what are the new paradigms to be developed?

In the past ten years, some of these changing paradigms have appeared in terms of new corporate entities in the natural product area dedicated to new pathways in drug discovery (Davidson et al., 1996). For example, the strategy of the former Shaman Pharmaceuticals was to focus only on plants used ethnomedically and they had the unique practice of sending an ethnobotanist and a physician into the field to better evaluate on-site the effectiveness of the ethnomedical preparations currently in use (Conte, 1996). Phytera, Inc. has a focus of enhancing metabolic processes in plant cell cultures to increase chemical diversity, trying to take full advantage of the genetic capacity of plants for secondary metabolism. It is important that different strategies are applied for natural product drug discovery which would be impossible to develop in a "traditional" pharmaceutical company. More such diverse approaches are needed, and one of these will described subsequently.

6. Evolving aspects of the discovery process

Initiation of a natural product drug discovery program involves a number of different phases, all of which have changed and evolved substantially in the past 5 years (Cordell, 1995b). These steps are: (i) defining the strategies; (ii) selection and collection; (iii) extraction and biological evaluation; (iv) dereplication; (v) isolation and structure elucidation; (vi) biological evaluation; and (vii) information management. Let us briefly examine each of these.

Important questions must be answered at the commencement of the research program (Cordell, 1993a). What organisms are to be used? Plants, marine animals, cyanobacteria, lichens, arthropods, epiphytic fungi? Where and how should the organisms be collected? How should the organism be extracted and which extracts should be tested? For example, is there any purpose in evaluating an aqueous extract of the organism if the program is only interested in developing nonpolar metabolites? Which bioassays should be used? What are the primary assays, i.e. those which are capable of evaluating large numbers of samples in a (semi) high-throughput manner? Which secondary assays might be used to evaluate actives from a mechanistic perspective, and therefore permit a prioritization for fractionation? Should the program focus

solely on the bioevaluation of a diverse selection of known natural products?

In considering the selection of the organisms, let us say that plant materials will be examined. Will the plants be collected in a locally random manner, i.e. what ever is available within a certain biodiversity-rich area? An alternative would be to use a phytochemical or taxonomic approach such that either certain chemicals, say isoquinoline alkaloids, are sought based on their chemotaxonomic distribution, or certain taxa are sought (e.g. *Taxus* species) because an important compound has been isolated and analogs are needed. In either case, substantial knowledge of bio- and chemodiversity is required. An ethnomedical approach, in which plants are collected based on their indigenous use for a particular indication, could be used. Yet another alternative would be to search for active metabolites based on an ecological approach where those plants which fill a particular ecological niche could be pursued, for example, those on which ants, or a certain insect, do not feed, or trees which do not allow an undergrowth to develop. The information-managed approach is a combination of several of these approaches which usually combines ethnomedical, biological, and chemical information (Cordell et al., 1991). Finally, there is serendipity, which is itself a very important and essential aspect of science. There are many instances in science where an experiment has led to an unexpected result which, in turn, opened up new opportunities for creativity (Roberts, 1989). In the discovery of biologically significant natural products, the recognition of vinblastine as an anticancer agent from the hypoglycemic plant *Catharanthus roseus* falls into this category (Taylor and Farnsworth, 1975).

In order for natural products to be more amenable to this bioscreening process, specific, selective procedures often need to be applied. For example, marine extracts may be pretreated by passage over a polyamide resin in order to absorb potentially HIV-interfering substances (Cardellina et al., 1993) after sulfated polysaccharides are removed (Beutler et al., 1993). For plant extracts, tannins and polyphenolic substances characteristically interfere with enzyme- and receptor-based inhibition assays giving false positive results. Consequently, they must be removed by one of the available methods (Loomis and Battaile, 1966; Wall et al., 1969; Tan et al., 1991). Another strategy which some pharmaceutical companies are using to make natural product extracts more amenable to screening is to develop a library of semi-purified natural product extracts by increasing the concentration of available, potentially desirable compounds.

For many years, various forms of dereplication were used in the evaluation of active microbial and plant extracts. These have included UV comparison, paper and thin-layer chromatography, mass spectrometry data base matching and bioactivity patterning. More recently, other dereplication techniques involving a combination of chemical/biological dereplication with database correlation have been introduced (Cordell et al., 1997).

Two other techniques involving natural products have been developed, and will become of increasing importance in the future as the need to enhance the chemical diversity of natural product sources continues. Natural product combinatorial chemistry involving actinomycin D conjugates has been described (Tong and Nielsen, 1996), and more recently, strategies involving combinatorial biosynthesis, particularly the ability to modify the metabolites of a polyketide biosynthesis pathway in *Streptomyces* have been delineated (Hutchinson and Fujii, 1995; Carreras et al., 1996; Kao et al., 1996; Yu et al., 1998). Some implications of these developments are described in more detail in the next section.

7. Discovery efforts for the near future

With the advent of combinatorial synthesis, some companies considered that this technique would answer the question of how to biologically evaluate a large number of compounds with the minimum of effort. Now that the euphoria has died down, the role of combinatorial chemistry has changed. It is now widely recognized that combinatorial chemistry probably does not belong as a part of the primary screening process, but has a very significant role to play when the pharmacophoric unit has been identified and potency, bioavailability, or some other attribute, requires optimization (Ecker and Crooke, 1995).

There is substantial interest at the moment in what is called combinatorial biosynthesis (Borman, 1998). In this process, genetic information about the specific biosynthetic pathway for a natural product(s) is altered in order to redirect the pathway to produce different metabolites, which can then be biologically evaluated. As mentioned previously this has so far been reported for aspects of the polyketide pathway (Hutchinson and Fujii, 1995; Carreras et al., 1996; Kao et al., 1996; Yu et al., 1998), where the synthases have been modified. A biotech company was founded to examine these options commercially (Borman, 1998). As more becomes known about the genetic aspects of other major biosynthetic pathways, one can imagine that many new products, closely related to present potent agents, might be formed in this manner. Previously, research which establishes biosynthetic pathways, and examines the enzymatic and genetic aspects of the processes involved were a badly neglected area for even modest investment (but see Donadio et al., 1991) by pharmaceutical corporations, or the federal government. Increased investment by both groups is now clearly needed to

aggressively pursue this opportunity. Although this process might yield fewer new pharmacophoric units of interest than the screening of diverse plant extracts, it will substantially enhance natural product chemical diversity and provide a number of other strategic benefits for the future.

As we have seen, there are multiple sources involved in the generation of samples for preliminary screening: libraries of synthetic chemicals, purified natural product extracts from marine and terrestrial sources, isolated natural products, and specific peptide and polyketide mixtures. At most pharmaceutical companies these libraries are being steadily expanded. However, the rate of expansion cannot compete with the increasing capacity for screening. Thus, one way in which chemical libraries are being enhanced is to exchange libraries with other companies, and for discreet organizations to serve as brokers for the provision of such collections of chemicals, including natural products. For biodiversity-rich countries this offers an interesting economic opportunity to provide preformatted extracts representative of their biodiversity.

Other procedures which might heighten the chemical diversity of natural product samples for biological evaluation include the use of modified extraction technologies, such as supercritical fluid technologies, the direct enzymatic or chemical modification of individual natural product extracts, and the use of selected natural product libraries based on a particular compound class.

The tremendous demand (Stinson, 1997) for chiral finished pharmaceuticals can only be met through semi-synthesis from chiral natural products, chiral total synthesis, or biocatalysis, the use of enzymes which can perform synthetic transformations to afford a high degree of enantiomeric excess. Biocatalysis is already proving to be very useful in the synthesis of a key intermediate for a new anti-AIDS drug, as well as for other key chiral intermediates (McCoy, 1999). In the future, there will be dramatic changes in synthetic methodologies wherein enzyme systems capable of highly directed chemical modifications will be responsible for multi-step synthetic protocols on a commercial scale. Such efforts will be complemented with sophisticated, fully automated solid and solution phase synthesis instruments for the development of compounds for screening. These facilities will also be critical in the area of "hit" optimization for computational chemistry.

Another area within biosynthesis where there are substantial opportunities for enhancing chemical diversity is the controlled potentiation of the enzymes of secondary metabolism in a given organism (plant, fungal or bacterial). We do not understand yet what is the totality of the biosynthetic capabilities which are present in even a single given organism, or how to modulate and potentiate them. Indications are that stimulant molecules (methyl jasmonate is certainly one) can have a profound effect on the enhancement of metabolic processes (Hashimoto and Yamada, 1994), and in the future there will undoubtedly be a group of chemicals or gene products which are routinely used to stimulate the full chemical diversity of metabolic capability. In addition, for plant and microbial cell cultures, there is the possibility to conduct the growth of the organism under altered conditions, and to examine whether the metabolism can be modified to produce a new range of metabolites for evaluation.

Yet another approach for the production of natural products which have not previously been accessible is to examine those microorganisms which at present cannot be cultured (Rouhi, 1999). It is estimated that less than 0.1% of the microorganisms in a soil sample can be cultured. The notion is to extract the soup of genetic information from the soil samples, paying no attention to the generating organism, and then insert the long DNA fragments which might contain biosynthetic information, into a bacterial artificial chromosome vector and then into a host organism (*E. coli, Streptomyces*, etc.) that can be cultured. If these systems can produce new molecular entities, they will certainly be attractive for the primary screening phase of drug discovery.

The human genome project will generate information on about 3 bn base pairs and about 117,000 genes. These data will have a very significant impact on the fundamentals of drug discovery, since it will undoubtedly foster a very large number of new disease targets which can be used for binding or inhibition or competition assays. Such strategies will be very useful in the bioautographic evaluation of natural product extracts through non-covalent binding strategies, followed by release and mass spectral detection; a modification of the pulsed ultrafiltration technique (Woodbury and Venton, 1998). Closely correlated with this technique is the need for future combinatorial chemistry and high-throughput screening to have systems which are capable of a higher level of quantitation in the primary screen, since quantitation of assay data is a primary concern for judicious decision-making regarding what constitutes an "active".

7.1. Creating balances

It has become increasing clear that, over all time since the origin of Gaia, the 20th century was the most destructive of earth's resources (Raven, 1988; Anonymous, 1992a,b; Principe, 1992; McDonald, 1997). While some efforts have been made to restore and replace utilized resources, for example, limited attempts at reforestation, the fact remains that the worlds' resources, renewable and non-renewable, terrestrial and marine, are being depleted at a staggering rate (Wilson, 1988; Akerele et al., 1992; Anonymous, 1992b; McDonald, 1997). The International Union for the Conservation of

Nature recently reported in their "Red List of Threatened Species" that one-eighth (34,000 species) of all flowering plants are presently threatened in 200 countries (Johnston, 1998), and 50% of bird species are anticipated to become extinct in the next 50 years (McDonald, 1997). United Nations estimates indicate that known oil reserves will last for only approximately another 70 years at even the present rate of usage. It will therefore be essential, for humankind to survive beyond this new century, for new balances to be created between humans and the biodiversity of nature. Accepting the Gaia hypothesis, that we are an integral part of a living earth, is a critical aspect in understanding our part in both the continuing destruction and degradation of the environment, and the restoration that must occur. It is crucial that we learn to see this as **our** destruction and **our** restoration. The legacy of what we are doing for our descendants bears consideration and contemplation, as has been offered previously (Cordell, 1992). Perhaps restoration, renewal and conservation are **the** best ways that we can show our gratefulness for what we have gained from Gaia. Let us examine some of the balances that must be restored as they impact the very limited area of natural product drug discovery.

One of the most important balances to be examined in the next 100 years will be that between conservation of the existing rain forests and their deforestation for grazing and crop lands. Not only are there important ecological, geological and meteorological reasons for maintaining the rain forests, we are aware of the tremendous biodiversity, and thus chemical diversity, which is stored. There is substantial agreement that the pool of plant and insect genetic density is greatest in these areas of the world. For example, there may be one hundred times the diversity of woody plants in a wet tropical rain forest in Costa Rica than in a temperate area in the central United States (Mooney, 1988). In addition, there is much untapped indigenous knowledge of the use of plants which is lost at the time of deforestation. Slashing and burning for grazing lands, or for short use as crop lands, depletes the soil and results in the irretrievable loss of many forms of biogenetic (and therefore chemical) diversity (Lugo, 1988).

Another balance to be considered in the future is that as more medicinal plants are brought (back) into mainstream prophylaxis and treatment regimens in the developed countries, the market growth must be balanced with the sustainable supply. Already there are instances, *Hydrastis canadensis* for example, where wild crafting of plants has resulted in the rapid depletion of natural stands of the medicinal plant to the point where it is now an imperiled species in 17 of 27 states where it is native, and is threatened in Canada (Concannon and DeMeo, 1997). In food and drug stores in 1998, sales of the top 13 herbal products were $640 million, up 58% compared with 1997. Sales for the first eight months of 1999 were about $489 million. Products such as saw palmetto, cranberry, and milk thistle have very rapidly rising sales (Landes, 1999). Is the industry deeply concerned about the conservation and sustainability aspects of these kinds of dramatic sales figures? At least one company has committed to the sustainable management of phytomedicinal products in the country of origin (King et al., 1999).

Synthetic medicinal chemistry in drug discovery places the human ego on the line to produce novelty and potency. Indigenous ethnomedical knowledge relies on the training and experience derived from generations of a deeply dependant relationship between humans and their local environment. Clearly, the latter is the more contactful with the notion of Gaia, whereas the former steadily depletes the available resources. In the past few years, greater emphasis has been placed on "green" synthetic chemistry, whereby reagents, including heavy metals, and solvents are reused and recycled where ever possible, or where previously unwanted waste products can be developed into useful synthetic precursors. The importance of this area was recognized several years ago with the development of the Presidential Green Chemistry Challenge Awards (Dagani, 1999). At the same time, there has also been a greater emphasis on the use of enzymes to perform certain key synthetic steps in the formation of biologically active target molecules (Turner, 1994). There is no doubt that fifty years from now synthetic organic and medicinal chemistry will be dominated by selective, high-yielding, reusable designed enzymes.

Another balance to be established is that between intellectual property rights and the burgeoning technology of drug discovery. This is an area of tremendous tension at the present time. We have already seen that pharmaceutical companies are attempting to develop libraries of natural and synthetic compounds and collections of biological diversity (plants, microbes, marine organisms, etc.) for present and future biological evaluation (O'Neill and Lewis, 1993). We have also seen that following the Convention on Biological Diversity, many countries are placing very strict limits, or developing tight regulations accompanied with very detailed procedures and protocols, for the provision of access to their biodiversity for the purposes of evaluation and potential commercial development. Those countries which can trust enough to provide access under reasonable terms of immediate and longer term compensation will find satisfactory economic reward and pharmaceutical development. Those countries which are overly restrictive and bureaucratic, or too demanding, will seriously lag behind in the development of their integrated pharmaceutical systems, and thus the cost of pharmaceutical entities for their people will be higher. Similarly, those companies which can offer and negotiate such

compensation packages in exchange for long term access and provision of materials will be welcomed as contributing globally to local economic growth and health care.

7.2. Creating alliances

International development in the search for new medicinal and biological agents now, and in the future, will require the development of numerous alliances. Indeed, as indicated elsewhere (Cordell, 1990, 1993a, 1995a), the future success of pharmacognosy totally depends on a very high level of collaboration and mutually beneficial partnerships. Only those individuals and groups who can set aside ego for the greater good of the whole will be successful. These alliances and collaborations may be either local or global. With the development of improved transportation systems for materials and people, and with the instant communications provided through electronic mail, global collaboration is almost as easy as working with a fellow scientist at one's own institution.

One of the earliest of the global collaborations which was of quite a high profile, and which engendered substantial interest, and in some quarters controversy, was that between Merck and INBIO in Costa Rica (Caporale, 1996; Sittenfeld, 1996a,b). Yet another example which reflects contemporary thinking is the relationship between Glaxo-Wellcome and the Brazilian biotechnology company Extracta, in which much of the discovery work will be done in Brazil, thereby building local infrastructure (Bonalume Neto and Dickson, 1999). Another form of the development of a global alliance is the International Cooperative Biodiversity program, which currently is a collaborative funding effort of the National Institutes of Health, the National Science Foundation, and the United States Department of Agriculture (Grifo, 1996; Rouhi, 1997). The second round of competition for these 5-year awards was recently completed. Another type of new commercial development agreement is that between Yellowstone National Park and the biotech company Diversa for the study of the chemical potential of geothermal organisms for drug discovery (Brennan, 1998).

There is a strongly prevalent thought that much of the research talent and resources in academia is unfocussed towards either local or global development. Industry, which is typically very oriented towards a profitable product, can substantially enhance the academic focus, and there are now several mechanisms for such collaborations. As mentioned earlier, one of these initiatives, with which we have been involved at UIC, is the National Cooperative Natural Product Drug Discovery program. In our case (Cordell et al., 1991; Cordell, 1993b; Cordell et al., 1993, 1994; Kinghorn et al., 1995) this program has evolved between an academic group (UIC), a research institute (Research Triangle Institute, North Carolina) and a pharmaceutical company (currently Bristol Myers Squibb, Princeton, New Jersey). This relationship has dramatically highlighted and prioritized our efforts to make decisions about which active plants to study further, and has allowed for a free flow of information and technology between all sites, together with the active participation of the National Cancer Institute, which also has a vested interest in our success.

That the global pharmaceutical industry has undergone a dramatic metamorphosis in the past 10 years is an understatement. Two major changes have occurred, the first is that numerous major amalgamations and take-overs have occurred which have significantly reduced the number of large pharmaceutical companies (Thayer, 1995, 1998a). This process is still ongoing, and it is widely held that ten years from now there may only be 5–6 large pharmaceutical companies operating globally. Secondly, there are now a very substantial number of small to moderate size companies, which are a part of the drug discovery process, with new companies beginning almost each month. This has led to unprecedented opportunities for the development of corporate partnerships, sponsorships and other relationships, which, in turn, has led to the phenomenon of the very large companies outsourcing for needed scientific strategies and technologies, and for the development of specific collections of natural and synthetic (usually combinatorial) libraries and bioassays (Myers, 1996; Borman, 1997; Thayer, 1998a). This activity is anticipated to expand rapidly in the future.

There are many current examples of very different types of outsourcing arrangements. One is that of a major company working with an academic institution to potentiate the technology which has been developed in academia where that company is given first-right of refusal to license any inventions (Blumenstyk, 1998). A second is a company serving as the developer of high-throughput bioassay screens, and then receiving compound/extract libraries from various other companies for evaluation on a confidential basis (Wierenga, 1996). Another is a small company working in a particular area of drug discovery science where the major company cannot develop that area and decides to provide major funding for the development within the specialty company. Several other arrangements have also been described (Wierenga, 1996), including those for drug development and clinical trials (Thayer, 1998b). The key is of course access to technology, and how to optimize that in such a rapidly changing environment where strategies, and the science and technologies which support them, are changing extremely rapidly and are very expensive. With the global pharmaceutical companies vying for the developed country marketplace, and internal research efforts being more highly focused

towards marketable projects, the speculative research, which often may be highly trendy and therefore viewed as risky, is initially being outsourced. As the number of major pharmaceutical companies diminishes discovery efforts as known today may be totally outsourced.

From a natural products perspective in a developing country environment, there are significant opportunities for economic and biodiversity development, if the relationships can be developed appropriately. This aspect was discussed in a recent contribution from this and several other laboratories: "The real benefit of establishing a biodiversity prospecting relationship is that it may provide the necessary stimulus or seed money to establish or improve in-country capacity to conduct research on genetic resources and support indigenous biotechnology and pharmaceutical industries" (Cragg et al., 1997b). Too often those who reach such agreements are being accused of biopiracy, because those who have the biological resources, and those who desire to evaluate that biodiversity, often have unrealistic expectations of appropriate shot-term and long-term agreements. In reality, this is a rapidly changing area, where agreements made one year may not meet the ethical standards of a subsequent year. More recently, our institution has completely restructured the benefits aspects in the basic agreement for permitting access. Thus we have developed a compensation strategy involving a program with a trust fund for the country of origin which assures that in the event of a royalty stream, the country of origin is compensated to a higher degree than is UIC. The future of such agreements, and therefore possibly the future of natural products chemistry in many developing countries, in addition to advancing health care, is based on negotiating the creation of value. It is also worth indicating that many synthetic natural product organic chemists should be interested in these issues since they relate directly to the intellectual challenge of the effective synthesis of biologically and chemically interesting molecular entities.

7.3. Creating value

It was Ralph Waldo Emerson (1803–1882) who asked "What is a weed, a plant whose virtues have yet to be discovered?". He was correct. One way to raise the awareness of the need to conserve biodiversity for future generations is to demonstrate the high levels of biodiversity and raise awareness of what assets plant materials have provided, and how their value can be enhanced through scientific evaluation.

For the success of any future enterprise which explores biodiversity there are two essential ingredients which require excellence: places and people. In many developing countries these ingredients are not optimized, for any of a number of different reasons. Thus, for a country which wishes to develop an integrated pharmaceutical system, or even a partially integrated system, development of those resources must be a high priority. Such developments should be included in the basic agreements which provide for access to the biome. What are some of these aspects of creating value?

Continuing to create value for biodiversity is absolutely essential for the future of civilization on Earth. Numerous authors have addressed the issue of how the environment can be protected from relentless destruction (Wilson, 1988; Anonymous, 1992b). One of the ways proposed is to create value in existing cleared land so that it is not necessary to continue to threaten biodiversity by clearing primary forests for their hardwoods. Thus, solid linkages must be established which conjoin preservation of the environment, research and discovery, and the development of an agro-industrial enterprise. For example, is it more beneficial long-term to import strategic natural pharmaceuticals or other natural products (essential oils, flavoring and perfumery components) than to encourage the development of the local capability to produce such materials? What are the short term and long-term issues involved? Is it possible that new industries might be developed based on local, sustainably grown and processed medicinal plants of global commerce which can reduce imports, and may increase exports? Without the establishment of studies of biodiversity, these and related questions cannot be addressed. One such example is the development of jaborandi as a crop plant in Maranhão, northeastern Brazil by E. Merck for the purposes of extracting pilocarpine (Pinheiro, 1997). Although this particular development initiative also raises a number of difficult issues of sustainable development of a threatened medicinal plant versus the livelihood of the local people. The relative merits of such developments should be established through integrated studies of the effects on local biodiversity as a component aspect of environmental impact.

There are areas of natural products research which are progressing rapidly and which could have a significant impact on local disease situations. One such area is that of transgenic plants. The production of known drugs in fast-growing plants through the introduction of the needed genetic apparatus for the biosynthesis of important secondary metabolites will be a critical use of the genetic aspects of biotechnology for local economic development. Thus the development of centers of excellence which could take advantage of such scientific opportunities will continue to be extremely important. An extension of this technology is the current impetus to develop vaccines that can be consumed through their production in crop vegetables (Lyons et al., 1996; Tackett et al., 1998). How can these technologies be transferred to a developing world? Will immunization against major global diseases (AIDS, malaria, tuberculosis, hepatitis C, etc.) be able to be

provided through genetically modified staple food crops (potatoes, rice, taro, etc.) in the future?

For the success of any scientific endeavor there must be first-rate scientists leading those programs and initiating new, economically significant programs. There are several facets to the production of appropriately trained, scientific personnel who can address the biological, chemical, and economic aspects of biodiversity. In some instances, strategic investment in the development of academic centers of excellence may be necessary. One model is to identify and aggressively support key laboratories in certain science areas or locations. Another is to select multiple groups in certain universities for major investment, while another is to develop collaborative relationships with academic institutions in developed countries or with pharmaceutical industry on highly targeted local areas of need. The latter approach may have the added advantage of providing both access to, and the experience of, collaboration and training programs in leading academic and/or industrial laboratories. Adequate access to personnel trained at the highest academic levels is crucial for economic and research development. Thus governmental support of PhD and postdoctoral programs, in country and abroad, which can provide the manpower to lead key research programs is essential. For those personnel whose PhD was obtained several years ago, but who may not have been afforded access to research program opportunities, postdoctoral training programs in outstanding laboratories are needed for future research development.

8. Challenges for the new century for natural products

At the conclusion of the most challenging century ever for Gaia (Wilson, 1988; Principe, 1992), it is essential that we assess how we can reconcile and restore our fragmented relationships with Gaia. Too often, and for too long, many in the human race have regarded themselves as separate from, and even "above", taking responsibility for the Earth. As Niebuhr indicates "The mastery of nature is vainly believed to be an adequate substitute of self-mastery" (Niebuhr, 1992). Indigenous peoples of North America and many, many other indigenous groups around the world who have evolved a deep respect for the Earth and its bounty had a quite different view. Chief Seathl, after whom the city of Seattle was named, in 1854, fearing the consequences of the white man's acquisition of his tribal lands wisely proffered, "One portion of the land is the same to him as the next, ... The Earth is not his brother, but his enemy, and when he has conquered it he moves on. His appetite will devour the earth and leave behind only desert" Maybury-Lewis, 1992). The deliberately set forest fires in the western Pacific rim area in 1997 and 1999 are testimony to this prophetic view. An isolated few have pioneered establishing the value of local biodiversity.

Perhaps it was the industrial revolution which gave us a sense that we needed and had power over the Earth. Perhaps it was the development of synthetic materials for buildings, cars, tools, and household goods. Perhaps it was the rise of synthetic organic chemistry which gave us a false sense of power for a myriad of amazing chemistry for a wide spectrum of purposes. In any event, contact with, and an appreciation and awareness of, and a gratefulness for the original source of those materials, and the fact that they are largely oil-derived, and therefore in limited supply, has been lost.

Pollution, of both the air and soil is apparently greatest in those countries which have the richest biodiversity and burgeoning populations. The Earth does not have the resources to support profligate waste for the next 100 years for our present 6 billion people, let alone for an anticipated population of over 9 billion people (Barney, 1999). Restoration, remediation and sustainability will be the essential global concepts for the new century as we continue to develop strategies which will provide for an enduring relationship with the Earth.

As the Convention on Biological Diversity has recommended for its signatories, the highest priority for the new century will be to preserve the rain forests and the oceans, and to maintain the ecological and biological niches that are apparently so critical in the production of secondary metabolites. Recognizing that we still know very little about vast aspects of the biological (let alone chemical) diversity on Earth, there is a continuing need to systematize the biodiversity, to catalog the species, and to establish the background ethnobotanical and ethnomedical information. Surprisingly, as we race to colonize space and other planets, there are no authoritative estimates for the number of species of plants, insects, fungi and marine organisms on this planet (Wilson, 1988). For example, only about 70,000 of 1.5–5 million fungi are considered to have been identified, and only about 800,000 of an estimated 20 million insects. There are an estimated 4693 ethnic groups in India, with relatively few of these studied for their sustainable use of plant materials for medicinal and biological purposes. In addition to the intellectual property issues which need to be addressed in order to pursue the investigation of ethnomedical information, it is apparent that many countries require more trained personnel who can evaluate this information. The majority of the botanic gardens in the world are in those countries which have the lowest biodiversity (Anonymous, 1992b). For the most effective development of the indigenous medicinal use of plants, there is a substantial need for the on-site involvement of, and cooperation with, physicians who can adopt both a holistic and a symptomatic approach to the treatment of disease.

There is a significant need to enhance drug discovery technology with respect to natural products, not for the next 10 or 20 years, because those drugs are already in discovery and development, but for the next 20–40 years and beyond. What are the areas for drug discovery where knowing the details of the human genome is either going to assist in developing new drugs or will specifically not be useful in alleviating disease, particularly for the majority of the world's population from developed countries who have very limited access to pharmaceutical agents? How will those health challenges be met? Clearly, screening of natural products and natural product extracts at the level of the gene for specific, clinically-related interactions is going to be extremely important in the future.

The suggestion has been made (Cordell, 1990, 1995a) that one way to enhance the local development of plants, and other biomaterials, is to develop genomics-based, in-field bioassays. Such assays would overcome one of the arguments that critics of biodiversity development raise, namely that all biological evaluations are carried out in developed countries, and that the results are not always returned to those providing the material. Such an approach might also promote the creation of value, or begin to validate traditional systems of indigenous medical knowledge.

We are steadily losing a number of plant species each day, and, as noted, one-eighth of all plant species are estimated by the International Union for the Conservation of Nature to be threatened (Johnston, 1998). Some of these lost species have never been catalogued, for the others they have never been evaluated biologically. An extremely high priority must therefore be to develop germplasm banks (botanic gardens, tissue culture collections, seed banks) in less-developed countries wherein plant materials under threat can be preserved for investigation for potential future development.

For trusting relationships to be strengthened and more highly collaborative between indigenous peoples or government or other agencies representing such groups, and those whose interest is in evaluating the biological potential, high priority must be given to developing assurances of intellectual property rights and compensation through long-term equitable agreements. Issues regarding the publication of ethnobotanical and ethnomedical information must be clarified or resolved so that the potential can be investigated in an equitable environment. The patenting of well-documented ethnomedical claims for individual plant materials must be halted.

Most importantly, as natural product chemists and biologists, we must maintain societal relevance. Without that we cannot expect financial or moral support. It is simply no longer conscionable for natural product scientists to be addressing issues which are not directly relevant to enhancing the use of the available biodiversity in a renewable manner for the benefit of humankind.

9. Conclusions

This, and several earlier reviews, have summarized how natural products from both terrestrial and marine sources have contributed substantially to drug discovery and the health benefits of humankind. With the advent of new approaches to drug discovery, it is manifest that innovative strategies will be needed for natural products to contribute their full range of chemical diversity to the discovery process for the future. It is also axiomatic that the development of plants and other natural materials for medicinal and biological purposes will need to be potentiated on a renewable basis in a local environment. Thus pharmaceutical companies should consider developing local resources and decentralizing selected aspects of their research operations globally.

We began this discussion with the concepts of gratefulness and constant transition. Let us conclude by indicating that this is an opportunistic time where we are clearly at a major crossroads in our relationships within Gaia. We can ignore the warning signs and continue to take the philosophical path of separation from the Earth. Or we can be grateful for the warning, and recognize that for many reasons, and the study of medicinal and biological agents is just one, we need to re-establish that we are an integral part of the biodiversity of an ecosystem which we have stressed substantially. Perhaps the conclusion of the human genome project, and the use of that information for drug discovery will assist in this. We will need the integrity and the vision to see that without such a restored relationship and a renewed balance with the Earth, our civilization will be primed to suffer catastrophic consequences.

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