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CHANGING STRATEGIES IN NATURAL PRODUCTS CHEMISTRY

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IN HONOUR OF PROFESSOR ANTONIO G. GONZALEZ

Key Word Index—Natural products chemistry; drug discovery; ethnomedicine; plant selection and collection; structure elucidation; biosynthesis, biological evaluation; strategy changes.

Abstract—Scientific strategies for the study of natural products from plants have changed substantially in the past few years for a number of reasons, including advances in technology, new molecules of substantial interest, changing ethical principles for organism collection and heightened awareness of the chemical and biological potential of the tropical rain forests. This review reflects on these changes and discusses, with recent examples, how they have impacted the conduct of natural products chemistry.

INTRODUCTION

In the past 10 years, approaches to the study of biologically active natural products have changed so dramatically that one is almost tempted to say that a new science has been born. Of course that it is not really the case, but certainly the quiet, almost silent, revolution that has occurred has for ever altered the way that natural products research will be conducted. In other presentations my colleagues and I have commented on some aspects of these changes, and in particular, how this has affected pharmacognosy [1–16]. Here, I would like to take a somewhat broader approach and examine how the combined impacts of changing biologies, technological advancements and the impact of new philosophies and a new awareness of biological diversity and its importance to the human race has modified the strategies by which natural product chemistry and biology are being pursued. In a previous article [17], Hamburger and Hostettmann reviewed the development of medicinal plant research in the period 1960–1990, as a celebration of the 30th anniversary of the founding of *Phytochemistry*. Several other articles have reviewed progress in natural product chemistry and the importance of natural products to the discovery of new biological and medicinal agents [10, 13, 15, 18 – 25]. In addition, *Natural Product Reports* published by the Royal Society of Chemistry provides regular coverage of many individual groups of compounds. This review complements and expands on certain aspects of those reviews and reflects, with the assistance of selected examples, on changes in the conduct of natural product chemistry which have occurred for political, scientific and technological reasons.

Some of the areas in which strategies for the conduct of natural product chemistry have changed include plant selection and collection, isolation techniques, structure elucidation, biological evaluation, semisynthesis, dereplication, and biosynthesis. Aspects of the tremendous changes occurring in each one of these areas will be mentioned, recognizing that each of these topics could be the subject of individual review articles or even books. Perhaps one of the significant aspects of the past 10 years has been the number of new journals devoted in whole, or in part, to aspects of the chemistry of natural products. These include *Phytochemical Analysis*, *Natural Product Letters*, *Phytotherapy Research*, the *Thai Journal of Phytochemistry* and the revived *International Journal of Pharmacognosy*. Established journals such as the *Journal of Natural Products* and *Phytochemistry* have steadily expanded. For example, in 1984 the *Journal of Natural Products* published 1088 pages in six issues. For 1994 the corresponding data were 1787 pages in 12 issues. Also noteworthy are the plethora of books at the scientific level, as well as many popular books on herbal remedies and the value of plants as medicinal and biological agents which are published each year. Some of the books reviewed in *Phytochemistry* and the *Journal of Natural Products* in 1994 are cited [26–46], including books which are devoted to broad-based studies on single compounds [45–47], the publication of papers from symposia on the chemistry of natural products [27, 30, 31, 44] and detailed compilations of natural products and their

sources [32–34, 36, 38, 42], something that would have been unheard of 10 years ago.

The burgeoning natural product literature has also spawned several current awareness services, including those of *Chemical Abstracts*, the Royal Society of Chemistry and *Phytochemical Analysis*. For natural product chemists, a significant development is having a source such as the *Dictionary of Natural Products* available in CD-ROM format, with a commitment to provide regular updates. Other database systems will surely follow. Coupled with desktop, essentially instantaneous, access to the Science and Technology Network (STN) of the Chemical Abstracts Service and the Grateful Med service of the National Library of Medicine, strategies for the acquisition of prior information have also changed substantially in the past few years. With more and more of the primary literature being available directly on-line, one can imagine that while strategies for publishing and accessing the chemical literature on natural products have changed substantially in the recent past, they will change even more dramatically in the very near future. The era of the printed scientific paper may be nearly at an end, replaced by a submission, review and publishing process which is totally electronic and instantaneously global.

This paper could almost be titled the 'Yin and Yang of Pharmacognosy', for what has happened in natural products chemistry in the past few years has been the establishment of a new balance, or rather several new balances. The very yang, masculine, pharmaceutical companies are still ever present, producing allopathic medicines at a steadily increasing cost, but now balanced, in a yin, feminine, way in many parts of Europe and the USA by a return to nature, to phytopharmaceuticals, and to natural remedies and cosmetics. The yang activities of collecting plants and other organisms without permission and consent are now mollified by a yin view in which indigenous rights are respected. Thus while the yang, high-tech, approach has certainly advanced both the pace and the sophistication of natural product chemistry, in the long run it will be the yin approach of sustainable conservation which will ultimately yield the greatest benefit for the Earth [48].

Besides a number of broad and quite general literature sources, including the popular press, articles in the 1994 issues of *Phytochemistry*, the *Journal of Natural Products*, *Phytochemical Analysis and Natural Product Letters* were specifically scanned for glimpses of these new strategies. Let us begin then at the beginning, for one of the most important issues that natural products chemistry may face in the years to come in access to biodiversity. Although this article is focussed primarily on plants and their derived products, many of the same comments also apply to the study of lower plants and microorganisms, as well as to marine organisms in all their vast diversity. One disclaimer: this brief review is not intended to be an up-to-date survey of the status of natural product drug discovery.

PLANT SELECTION

The six approaches to the selection plant materials for study have been described elsewhere [6, 7, 10, 15]. Here it is sufficient to indicate that of these approaches, the locally random, the taxonomic, the ethnomedical, the phytochemical, the information based and serendipity, two in particular, the phytochemical and the information based, essentially for the same reason, have been modified in recent years. That reason, of course, is the introduction of the personal computer and the resultant desk-top access to large databases which can be processed in a myriad of ways.

One example of such usage was described in connection with our discovery program on plant anticancer agents [7, 8, 11, 12, 14]. But theoretically, the same strategy could be applied for programs aimed at drug discovery in any one of a number of therapeutic areas, including antifungal, antiviral, analgesic, antimalarial, antibacterial, antidiabetic, and cardioactive agents. Such a strategy relies on the ability to have available a database which has three sets of information: (i) a list of those plants for which there exist ethnomedical reports of biological activity, (ii) a list of those plants for which there is either *in vitro*, *in vivo* or in human biological data regarding activity, and (iii) a list of the sources of the natural products which show activity in this therapeutic category or in any one of the bioassays mentioned in (ii).

In a discovery program, there is rarely a desire to reisolate known active metabolites which do not have the potential to be developed further. Thus, the goal is established of matching these data bases in order to establish a group of plants, or other organisms, for which there are both ethnomedical and biological data available, but from which no active principle has been isolated. If this list of plants is long, and potentially beyond the fiscal resources needed to collect the whole list, secondary criteria can be applied, including, the incidence of multiple usages around the world, clinical versus biological data and the endemicity, uniqueness or phytochemical knowledge related to a particular genus. From such a study, a list of potential plants to be collected may be whittled down from several hundred to a more manageable 50 or 100 high-priority, targeted plants for collection. Recognizing that such collection programs are very expensive and more time consuming that locally random collection, it is common that all available plant parts, other than solely the part indicated from the ethnomedical usage, are collected.

Farnsworth appreciated that the compilation of literature data was important for the future of natural product chemistry, and began in 1969 to publish a current awareness journal *Pharmacognosy Titles* on a monthly basis. In 1975, it was recognized that these data should be computerized in a more accessible and malleable form, and NAPRALERT was born. This system has the ability to do analyses of the type described above, and indeed has been used for that purpose in the development of several drug discovery programmes both by our group at the University of Illinois at Chicago, as well several pharmaceutical companies and the World Health Organization. NAPRALERT contains ethnomedical, chemical and biological information from more than 115 000 scientific research articles representing 129 000 chemical com-

pounds, and over 48 000 plant, marine, microbial and animal species. The database is available through a number of global information networks, including the Scientific and Technical Information Network of the Chemical Abstracts Service. It is extensively used by the pharmaceutical and herbal industries, as well as by government agencies and individuals in academic settings all over the world.

Since more than 80% of the world's population use plants as their primary source of medicinal agents [49], it is not surprising to find that in many countries of the world there is a well-established system of traditional medicine, whose remedies are still being compiled. In some instances, such as the Chinese, the Ayurvedic or the Kampo systems these remedies are well documented, and many are commercially available, being produced as lyophilized extracts in facilities comparable to those being used for the production of allopathic medicines. In other cases, traditional remedies are closely guarded secrets held only by the shaman or curandero and passed on solely to tribal apprentices [50]. While alarm at the rate of disappearance of traditional healers and their apprentices has been expressed [51]. it is also true that until the late 1980s there was relatively limited interest in the developed world in such indigenous knowledge providing only minimal assistance to developing countries to preserve, collect and systematize such knowledge. The recent substantial interest in natural products has resulted in an increasing respect for this knowledge, particularly as products based on some of this knowledge have been introduced in the developed countries. The numerous issues surrounding biodiversity prospecting have been described in several books [52–55]; aspects will be discussed subsequently.

The area of science which has recently come to the fore, as at least an alternative way to discover biologically active natural products from plants, is ethnobotany, whose leading journal is the *Journal of Ethnopharmacology*. Ethnobotany has evolved rapidly in the past few years, and several authors have reviewed aspects of these developments [56–59]. The CIBA Foundation recently organized a follow-up symposium [60] entitled '*Ethnobotany and the Search for New Drugs*' demonstrating the substantial rise in activity and acceptance of using an ethnobotanical approach for drug discovery. In this symposium, the approach of Shaman Pharmaceuticals has been described by King and Tempesta [61] and elsewhere by King [62]. Before an expedition, regional studies are conducted on the epidemiology, traditional medicine, culture and ecology of the people and their environment. In the field, ethnobotanists and medical doctors work together in presenting specific disease case descriptions having visible symptoms to shamans. Working initially with local translators, once the shaman has recognized the disease, the plant material(s) and their method of preparation used for treatment are recorded and the plant collected. Further observations are made when the physician assists the shaman in providing health care to the local people. Shaman has focused on the establishment of long-term relationships with various indigenous communities and has established policies which provide for short-, medium- and long-term benefits to these communities through a conservation organization: The Healing Forest Conservancy. One of the goals of returning resources to the community is to establish sustainable supply and extraction industries, which is considered a vital aspect of economic development. Besides returning results of biological experiments to the communities, such partnerships also involve assistance in the conservation of biological and cultural diversity, thereby forging a strong linkage between the discovery and sustainability.

In 1989, Phillipson and Anderson [63] reviewed the contribution that plants had made to Western medicine, using as an example the application of various alkaloids in current medical practice and also citing the proceedings of several meetings which focussed on drug discovery from traditional medicines. Their conclusion was that this area deserved 'to be pursued rigorously'. However, the fact remains that many scientists in pharmaceutical industry offer scant respect for the possible effectiveness of an antimalarial remedy of a hill-tribe in Thailand.

Cox and Balick, both former students of Richard Schultes, who is regarded as 'the father of ethnobotany', have also described their approach to ethnobotanically based drug discovery [64]. They discuss the validity of searches based on ecological niches, such as absence of predation, or use as fish poisons or in blow darts, and the approach of working in regions with tribes which have powerful healers. It has long been established that such information sources provide a much higher 'hit rate' for plant extracts than random screening, and Cox and Balick have independently confirmed this. They go on to describe how to select a tribal society with which to work, biological diversity, stability of the society, tradition of healers who pass their knowledge on through apprentices. Cox has worked in Samoa with the herbalists or taluasea and describes how it may take weeks, months or even years to establish relationships, learn local customs and language and the uses of the plants in healing. Permission is sought to collect plants which are then dried and shipped. Cox has described how from the Samoan plant *Homalanthus nutans*, the phorbol derivative prostratin was discovered as possessing good anti-HIV activity, following on the lead of a plant used locally for yellow fever. Compensation to the Samoan people has been assured through royalty commitments, and a foundation has raised funds to establish rain forest preserves in Samoa for the sustainable use of medicinal plants by future generations. Balick has described his work in Belize which has led to the founding of an ethnomedical forest preserve, managed by a local association of traditional healers. Such steps are very empowering to local peoples who typically feel powerless in the face of logging companies and ranching combines.

Holland [65] has suggested that an alternative approach to drug discovery would be to scan ancient texts for clues to new biological agents, as part of a collaboration between classicists (Greek and Latin) and scientists. Additionally, as Bruhns has related [66], even ancient monasteries, such as one in Soutra, Scotland, the site of

a medieval hospital, may yield clues to the use of medicinal plants (opium, hemlock, henbane and even ergot). Though the finding of medicinal plants in grave sites dates back to at least the Middle Paleolithic period, 60 000 years ago [67].

PLANT COLLECTION

One of the first decisions that anyone planning a programme for natural product discovery research and development faces is the source(s) which will be used. This decision, together with decisions regarding extraction processes and biological assays are the fundamental framework of a discovery programme. For many pharmaceutical companies, the decision has been simply to acquire biodiversity in order to broaden the chemodiversity of available structures. Their linkages for natural product screening are through chemodiversity obtained either from existing in-house or purchased chemical libraries, or through combinatorial libraries [68–70]. Thus, a random collection of say 10 000 different plants may represent 50 000–100 000 different natural product structures. Like the libraries of purified chemicals, these samples become part of a bank of samples which can be put into 96-well plates for biological analysis in the same way that 50 000 synthetic chemicals can be screened. For most pharmaceutical companies, until a lead is identified and more sample required, the source is irrelevant. This point will be expanded upon subsequently.

Once a natural product lead is identified through a random screening process, different strategies come into play. For example, if a lead were identified from the genus *Bupleurum* in a particular enzyme-based assay, and additional testing revealed substantial *in vivo* activity, a decision would have to be made as to how this would be pursued; (a) chemically with respect to sourcing either the natural product or an active derivative thereof, or (b) chemically with respect to a total synthesis/medicinal chemistry initiative for either the whole structure or its pharmacophore. In the event that the lead compound was not amenable to effective synthesis, alternative sourcing, involving the collection and study of other members of the genus, or other plants known to contain similar metabolites would be initiated. If these approaches fail, semisynthesis of related, more abundant derivatives, or alternative cultivation strategies, such as establishment of plantations or tissue culture would be considered.

A recent example where the factors of the development of human medicines and protection of the environment have been played out in a quite open forum has been the development of taxol (now called paclitel) as a clinical entity for the treatment of cancer [46, 47], following the demonstration that it operated by a different mechanism of action than any other known cytotoxic agent [71–74]. Numerous reviews have discussed the discovery, the chemistry and the biology of taxol [75–85], a compound first described as a cytotoxic agent from the Western Pacific yew, *Taxus brevifolia* by Wall and collaborators in 1971 [86]. In Phase II clinical trials, taxol was found to have excellent activity against ovarian cancer [87], but the problem which had loomed earlier, supply, now came to the forefront. In order to obtain 1900 g of taxol, it had required the felling of 6000 trees to acquire 27,300 kg of the bark from this very slow growing tree; 25 kg of taxol were to be required for the multicentre clinical trials. Clearly, if taxol were to become an approved agent, as it now has, this was not an environmentally acceptable source [88, 89], indeed some estimates projected such a source of supply would last for only 5–10 years [77]. New strategies directed towards the continuous supply of taxol were needed.

An unprecedented meeting was organized by the National Cancer Institute in Washington, DC in June, 1990 to attract commercial and academic participants to the program and develop a plan. Four possibilities for supply could be considered: (i) find a better source for the supply of taxol such as a different species or a cultivar of *Taxus*, or a different plant part or cultivation conditions, (ii) semi-synthesis of taxol from a more abundant precursor, (iii) total synthesis of taxol, and (iv) tissue culture production of taxol or a close relative [90]. All of these routes have been attempted, with success being achieved in only one: semisynthesis. Some might argue that total synthesis is also a success, and indeed three total syntheses have been achieved [91–96], and are monuments to the evolution of synthetic organic chemistry. But these routes, and with several others underway, will not supplant semisynthesis of taxol from 10-deacetylbaccatin III [97], or even afford derivatives whose biological significance can be evaluated for potential drug development.

The increasing interest in taxol and its derivatives, coupled with issues of supply have prompted a number of studies related to the storage of *Taxus* plants. Sabin and co-workers [98], for example, analysed seven taxanes in extracts from the bark and foliage of *Taxus brevifolia* during the course of a year. Taxane levels tended to increase during the late spring and during the summer. Levels of baccatin III and 10-deacetyl baccatin III were maximal in the late fall.

Taxol clippings have also been suggested as a potential source of taxanes, and Nair and co-workers have studied the effects of post-harvest storage on taxol content in *Taxus* × *media* 'Hicksii' [99] and found that yields were slightly higher in the biomass after about 10 days of storage, particularly at 22°. Croom and colleagues looked at clippings of dried *Taxus* biomass on a monthly basis over 15 months [100] and found that dried clippings could be stored without loss of taxol and that fresh clippings also maintained their taxol level over 10 weeks when stored under refrigeration. These are all important steps in considering future availability of the drug.

The analysis of *Taxus* samples for taxol and taxane derivatives has attracted substantial attention as the search has continued for either high yielding strains of *Taxus* containing taxol or its derivatives. Among the many techniques which have been applied are fast-atom bombardment mass spectrometry [101], matrix-assisted

laser desorption mass spectrometry [102], as well as thermospray [103] and electrospray [104, 105] techniques. An enzyme-linked immunosorbant assay (ELISA) has also been described, but cross-reaction with the related compound cephalomannine was observed [106]. Two groups have now reported the use of tandem mass spectrometry to address this analytical issue [107, 108]. Hike *et al.* [107], for example, applied tandem MS–MS with an internal standard of trideuterated 10-acetyl taxol, and found that extracts could be analyzed at the picomole level with a relative standard deviation of 17% or better in 5 min or less. In addition, these samples were analysed by HPLC which was also effective in the high picomole to low nanomole range of detection, but the sample analysis was longer (40 min).

The Bristol–Myers Squibb group [108] used an integrated analytical system to profile 18 naturally-occurring taxanes. The system consisted of reversed-phase HLPC with photodiode array detection coupled (20:1 effluent split) to the ionspray (nebulizer assisted electrospray) interface which generated abundant $(M + NH_4)^+$ ions as the base peak for each taxane. The patterns generated by the collision-induced dissociation of the $(M + NH_4)^+$ ions were then used to provide a template for identification of substructures. The correspondence between the LC–UV profile and the LC–MS total ion chromatogram was impressive in showing the power of these combined techniques to determine the potential nature of taxanes in examining biomass samples, process intermediates and unfractionated active samples.

The search for new agents continues, now using very different approaches. Several years ago, the National Cancer Institute described the establishment of intramural drug discovery programs for both cancer and AIDS, whereby samples from both the marine and the plant kingdoms would be collected, extracted and screened in the selected assays [109–112]. In this program, locally random collection of samples occurs, and for the collection of plants, the world has been divided into three areas, Africa, Central and South America and the Western Pacific rim. Three groups, the New York Botanical Garden, the Missouri Botanical Garden and our group at the University of Illinois at Chicago, led by Dr D. Doel Soejarto, have been contracted to provide samples. When a lead is identified and scale-up is needed, the original supplier is contracted to acquire additional material for large scale isolation.

Pharmaceutical Companies who are also accessing plant materials on a broad basis, now routinely use botanical gardens and taxonomists at universities to assist them in their sourcing of plant biodiversity, as well as specialized groups experienced in the acquisition of various marine samples, including those in deep water, where submersibles are required. This is a major strategic shift from the way that samples were collected when the natural product collection programmes focused more on the evaluation of microorganisms, particularly those derived from soil samples. Under these circumstances, it was common for the pharmaceutical company to ask its employees, whenever they travelled on business to a new location or went on vacation, either locally or abroad, to bring back several soil samples which could be sent to the microbiology department for culturing, selection, identification and eventual screening and storage. Numerous antibiotics were discovered in precisely this manner [113], without consideration of compensation or acknowledgement of the source. For reasons which will become immediately apparent, this approach to obtaining microorganisms is no longer acceptable; those reasons relate to intellectual property rights.

INTELLECTUAL PROPERTY RIGHTS

No other area of natural products chemistry (not even the story of taxol!) has received as much attention in the past few years as intellectual property rights (IPR). Although perhaps not yet widely appreciated by many natural product researchers, IPR and the Convention on Biological Diversity which emerged from the Earth Summit held in Rio de Janiero in 1992 [114], may, in the long-term, be one of the most profound steps ever taken in natural products chemistry. Heads of State, political leaders, environmentalists, activist groups and scientists witnessed a meeting at which, with the exception of one nation, 153 countries agreed that beyond personal interest, the health of the planet and the conservation of its resources, particularly its biodiversity, were the most important single factors for the long-term survival of the human race.

The Convention commits the signatories to substantive action in numerous domains within their territorial boundaries [115], including the development of national plans, strategies or programmes for the conservation and sustainable use of biodiversity; establishing an inventory and monitoring of the components of biodiversity and of the processes adversely impacting it; the development and strengthening of mechanisms for biodiversity conservation; the restoration of degraded ecosystems; the preservation and maintenance of indigenous systems of biological resource management; the equitable sharing of benefits with such communities; and the integration of biodiversity concerns into national decision making. The new global awareness provoked as a result of the Convention and its outcomes has substantially heightened public knowledge of the issues with respect to the tropical rain forests of Asia, Australasia, Africa and Central and Southern America. With that has arisen a new awareness in many countries that biodiversity is indeed valuable, that a country does have the right to protect access to it and the right to demand compensation for such access [53, 116].

In the long term, conservation of genetic resources and continued, controlled access to these resources both terrestrial and marine are critical for the future development of natural products chemistry. The future health and welfare of the human race may well depend on the appropriate exploration of these genetic resources [22, 48, 117].

One other aspect of plant collection which has changed as the selection and testing strategies have evolved is the scale of operation of the collection programme.

When *in vivo* biological evaluation was the primary screening modality, it was necessary to collect initially 5–10 kg of dried plant material so that a reasonable quantity of extract could be available to allow testing up to 200 or even 400 mg kg^{-1} in a rodent model. As more and more highly selective *in vitro* assays have been developed, the quantities of dried plant necessary for total collection, including storing material for the early part of the next century, has dropped to 50–500 g. The ecological impact of this reduced collection requirement should not be underestimated. In addition, these trends have been extended to the plant parts which are collected, i.e. collectors will usually not collect bark material from living specimens, if this will risk survival of the tree, and only very limited parts of total root systems are collected.

Environmental issues are also very much involved in a situation regarding a compound being evaluated as a potential anti-HIV agent. Calanolide A was isolated by a group at the National Cancer Institute, together with several related derivatives, from the fruit and twigs of the tree *Calophyllum lanigerum* var. *austrocoriaceum* collected in Sarawak, Malaysia [118]. The compound displayed quite potent activity in the whole cell HIV-1 assay and was considered a good candidate for development. However, when Soejarto and colleagues returned to the tree that had yielded the original sample it had been cut down, only the stump remained. Four further collection trips seeking both neighboring trees of the same species and in the same genus have thus far failed to yield calanolide A [119]. Although some related compounds have been isolated from *Calophyllum teysmannii* var. *inophylloide* [120] and *Calophyllum inophyllum* [121], as yet, none has shown the activity profile of calanolide A. Now seedlings of *C. lanigerum* have been established at the Botanical Research Center in Sarawak, and the provincial government has banned logging of the species [122].

Another group of compounds which is proving to be of great interest from many different perspectives are the michellamines [123], a group of dimeric naphthylisoquinoline alkaloids possessing anti-HIV activity [124, 125]. The compounds were isolated from a new species of *Ancistrocladus*, now named *A. korupensis* after the Korup National Park in Cameroon where the trees yielding these alkaloids were first isolated [126]. Several challenges have dominated work on these compounds including defining the three types of stereochemistry (four centres, two biaryl axes and a configurationally labile axis) in each alkaloid [124, 127], the ability of certain of the alkaloids to interconvert [125] and their partial [128] and total, stereochemically controlled synthesis [129, 130]. The compounds appear to act by two different mechanisms, reverse transcriptase inhibition and inhibition of syncytium formation [131].

PRIORITIZATION

Within a biological agent/drug discovery programme searching for leads based on natural product sources, there are always bottlenecks; points at which the rate of flow exceeds capacity. It is now becoming apparent that selection, collection, extraction, and biological evaluation are no longer impediments to the flow of samples through the discovery pipeline; but that bioactivity-directed fractionation certainly is. Depending on the 'hit rate', a concept which will be described and discussed subsequently, there may be a few (10–20) or many (several hundred) samples which show 'activity' in a given bioassay screen. Isolation and structure elucidation of the biologically active principle requires the skill of an isolation chemist, maybe several. Since this step is so labour intensive, a chemist can work on only a limited number of active samples at the same time, maybe 10 if the activity is well-concentrated into limited fractions, or six if the activity is spread though several polarity/chemical groups. Because of this limitation, one is immediately faced with developing a method, or a series of secondary criteria, to the selection process. In many large pharmaceutical companies, these strategies include, applying a secondary screening process using additional bioassay(s), evaluating the spectrum of response in the 40–50 primary bioassays currently operating in order to establish a unique, or a multiple level of interest based on selectivity or potential mechanism of action. Chemotaxonomic or ethnomedical information can be applied to eliminate species which either have been well-studied previously or which are likely to yield known active metabolites.

The preference, almost requirement, of novelty of structure associated with biological activity in order to establish a patent position results in other strategic choices. For at this point many groups apply various dereplication strategies. In terms of time to isolate a novel active compound and more importantly, to conclude one study and move on in a timely manner to the next active plant, companies have developed sophisticated, literature surveillance, HPLC-linked mass spectrometric systems tied into databases of natural products whose mass and UV spectra have been characterized.

An extension of this process, where equipment is now being marketed, is to access the HPLC effluent from such a system and after concentration into 96-well plates, evaluate the separated extract biologically, and, through database access to natural product libraries, begin preliminary identification of natural products at the earliest possible screening stage. This strategy serves a many-fold purpose. Firstly, it allows the original activity to be confirmed, possibly in a secondary assay. Secondly, it may illustrate whether one or more compounds, or types of compounds, are responsible for the biological activity. Thirdly, when the activity is associated with a given fraction of the chromatogram, where a dominant compound is present, it may be possible to identify that compound directly. The process has been described as consisting of three phases, a separation phase, an information gathering phase and an information management phase. Following HPLC gradient separation, the eluant is passed through a UV–Vis diode array spectrometer and the stream split unequally (approx. 49:1). Post-column, the smaller stream is treated for either

positive and negative ion production in the electrospray interface of a mass spectrometer. The larger stream is recovered into 96-well plates and the sets of plates are bioassayed. The datasets of UV–Vis, positive and negative ion spectra and the bioassay result localize the activity to a portion(s) of the HPLC chromatogram. Knowing the botanical source, the compounds of selected mass may be searched in the database for that plant or a related species, and the mass and UV spectra of candidate compounds compared. Such technology is now routinely being used in our laboratory for the evaluation of samples displaying anticancer, cancer chemopreventive and anti-HIV activity [132]. In the event that an active extract affords a separable series of unknown active compounds, this extract is considered to be of high priority for large-scale (100–1000 mg) fractionation. We believe that this procedure will become routine in many drug discovery laboratories in the near future.

Corley and Durley of Monsanto Company [133] have provided an overview of the databases available for dereplication including the *Dictionary of Natural Products*, *Bioactive Natural Products Database*, DEREP, MARINLIT, and the *Marine Natural Products Database*, as well as the services of selected files of STN. In this way, information services, both for literature as well as the substructure searching, can often be brought to the bench chemist rather than being in the hands of an information specialist. They have estimated that for each natural product dereplicated a savings of approximately US $50,000 is generated in isolation and identification time, which can then be used for the discovery of novel bioactive compounds.

There are also other purposes related to dereplication studies. One of these was uncovered recently by the National Cancer Institute in their anti-HIV screening program [134, 135]. About 15% of the extracts from plants, cyanobacteria and marine invertebrates and algae showed activity in the primary whole cell anti-HIV screen. Such a high percentage indicates that either the criteria for activity, the 'hit rate', is set too high or that there is a recurring group of active compounds. The NCI group discovered that in this particular case it was the latter, and traced the activity to the frequent occurrence of sulfated polysaccharides, which are known to possess anti-HIV activity [136]. This led to the development of a dereplication protocol for the removal of anionic polysaccharides by precipitation from an aqueous solution with an equal volume of ethanol [134] prior to biological evaluation.

In a way, this situation parallels the experience that numerous research groups around the world have recently experienced with the group of compounds known as tannins. Both the anthocyanin-derived, as well as the condensed tannins contain numerous phenolic hydroxy groups. These compounds have the ability to bind to diverse proteins and enzymes leading to inhibition of the enzyme activity. If that inhibition is what is being measured in the particular assay, and yet tannins are deemed to be not of interest for development, a 'false-positive' response has been obtained. Given the very wide distribution of tannins in the plant kingdom [137], it has been necessary to devise a number of techniques to eliminate tannins from the screening process. Some strategies incorporate this as a first purification step to all extracts after the extract is obtained, other strategies include this only for 'actives', which are then detannified and retested. Several procedures are available for detannification [138–141], and the choice is often one of personal preference. There has been no definitive study as to which procedure, caffeine precipitation, polyamide chromatography or PVP complexation, is the most efficient, nor has there been a study of the selectivity of these procedures for the various classes of polyphenolic compounds (flavonoids, xanthones, lignans, etc.) which might also be removed in these procedures [135].

The NCI group has extended this strategy to a more general approach for aqueous extracts [135] in which a series of solid phase extraction cartridges is used for dereplication. After a 50% aqueous ethanol precipitation to remove the polysaccharides, the supernatant is passed through a polyamide column to remove tannins and then through Sephadex G-25, and selected bonded-phase cartridges (C_4 and C_{18}). Four fractions were tested and different elution profiles retaining anti-HIV activity were observed. This technique complements some other chemical screening approaches [142, 143], as well as traditional phytochemical screening [144], which do not concurrently focus on biological activity.

Cucurbitacins are well-known as cytotoxic agents, and cucurbitacin E is being evaluated in various *in vivo* xenograft models as a potential agent for clinical development. Consequently, there is a need to identify plants which may contain these compounds as active metabolites at an early stage in the isolation process. The COMPARE program [145] permits the recognition of patterns of cytotoxicity in the NCI cell line panel, and the NCI group has recently reported the use of this program in the dereplication of extracts of *Iberis amara*, *Begonia plebeja* and *Gonystylus keithii* for cucurbitacins [146]. Coupled with literature analysis, this permitted decisions to be made regarding whether to conduct a detailed bioactivity-directed fractionation or simply fractionate for the anticipated active principle(s).

ANALYTICAL TECHNIQUES

The new journal *Phytochemical Analysis* is dedicated to the description of new, analytical research techniques and their application in the analysis of natural products. Hence this is a good source of numerous techniques which may have broad application beyond their original use. In addition, there are regular reviews of important analytical techniques once they have become well-established in the field and are widely available.

Chromatographic techniques

The ability to separate and purify steadily decreasing amounts of natural products from complex matrices has been a major factor in the burgeoning of known natural products in the past 15 years. Numerous new techniques

have been introduced including droplet counter-current chromatography (DCCC), rotation locular counter-current chromatography (RLCC), and centrifugal partition chromatography (CPC). In addition, the advent of reverse-phase strategies for chromatographic separation, either through TLC or HPLC has afforded the natural product isolation chemist a very diverse range of techniques which might be applied to individual challenges of separation. Several years ago, Hostettmann and coworkers reviewed the progress in CPC technology and its use for the analytical and preparative separation of diverse natural products [147]. In addition they compared the effectiveness of CPC, DCCC and RLCC for the separation of a series of flavonoids and found the former technique using a multicoil system to be the most effective [147]. The same group has also reported on a bioautographic method for the detection of antifungal (*Candida albicans*) compounds [148] to parallel a previously described antibacterial technique [149].

New techniques for isolation using other forms of chromatography are also under development. For example, Moriyasu and colleagues have described the use of ion-pair extraction and ion-pair high performance liquid chromatography using perchlorate as the ion-pair reagent for the concentration and preparative separation of the quaternary quinoline and isoquinoline alkaloids of *Zanthoxylum usambarense* [150].

Chiral procedures

Most natural products occur in only one enantiomeric form (e.g. mono-sesqui-, and triterpenoids, as well as steroids). Other natural products may occur in either enantiomeric series (e.g. diterpenoids), while in other cases one enantiomeric series dominates, while the antipode may occur in perhaps a different type of organism (e.g. amino acids, sugars, certain alkaloids). Since the recognition of chirality, non-destructive methods for resolution of enantiomers, or the ability to define enantiomeric purity have been needed by natural product chemists. More recently, this need has spread to the biological area, where, as more enzyme and receptor interactions have been probed at the molecular level, it has become clear that enantiomeric compounds can be expected to differ substantially in their biological activity [151]. In 1992, the U.S. Food and Drug Administration issued guidelines regarding the marketing of chiral drugs which left pharmaceutical companies the choice of whether to develop such drugs as racemates, single enantiomers or enriched mixtures. For most companies the decision was to commit to a single enantiomer, resulting in a new facet to the biotechnology industry dedicated to the improvement of chiral technology [152]. The implications for natural products chemistry are many-fold regarding both the enantiomeric purity of a particular isolate, which may be difficult to define in the absence of a purified enantiomer, as well as the biological aspects of the isolate. Thus, interest has grown in the synthesis and evaluation of *ent*-natural products.

Brossi has examined a number of interesting alkaloids in this respect, including colchicine, physostigmine and morphine [153], and our group has looked at the biological activity of the atropisomers of gossypol [154,155]. In the latter case, the (−)-form acts as a male contraceptive agent, whereas the (+)-form is devoid of comparable activity. Our work was critically aided by a collaboration with Pirkle at our Urbana campus, who at the time was developing various chiral stationary phases [156].

Since that time numerous types of chiral stationary phases have been introduced which have been characterized into five types based on their solute-chiral stationary phase bonding interactions: the helical polymer phases, the ligand exchange columns, the protein phases, the chiral cavity phases and the donor–acceptor type columns. For the separation of natural or synthetic racemic, or partially racemic mixtures, for investigating the enantioselectivity of molecular recognition from a biological or a chemical perspective, it is clear that such chiral systems have become an integral and rapidly evolving aspect of natural products chemistry. Strategically, the synthesis of a racemate which can be separated into its various stereodiscrete forms, which can then be evaluated biologically offers an attractive approach to potentially optimizing biological activity for a given structure.

Chiral procedures using modified cyclodextrins (e.g. octakis-(2,3-di-*O*-acetyl-6-*O*-*tert*-butyldidmethylsilyl)-γ-cyclodextrin and heptakis(2,3-di-*O*-methyl-6-*O*-*tert*-butyldimethylsilyl)-β-cyclodextrin) are also available for gas chromatography and have been applied to the separation of monoterpenes [157] and apple butanoate esters [158], respectively. More recently, this work has been extended to a complete stereoanalysis of Buchu leaf oil which is used in various aromas and fragrances [159]. Such analyses may in the future become a very important way in which essential oils are evaluated and graded for their sensory qualities. Similar comments might also be made about the flavours of fruits. The same group, for example has looked at the chiral separation of the butanoate esters of various apple varieties using enantiogas chromatography isotope ratio ms [160].

The need for the analysis of phytopharmaceuticals, as well as traditional medicines and various ethnomedical preparations, has continued to spawn numerous improvements in the analytical techniques for the standardization of the active principles. Quantitative tlc has recently been used [161] for analysis of the iridoids of *Linaria* from which acetylpectolinarin is used as a cardiostimulator, and a rapid HPLC system has been described in order to analyse the valepotriates in hairy root cultures of *Valeriana officinalis* var. *samucifolia* [162]. A Chinese group [163] has applied SIMCA pattern recognition for the evaluation of extracts of *Tripterygium wilfordii* and *T. hypoglacum*, which are used for their anti-inflammatory activity. Using infrared data from various wavelength channels, it was possible to classify the roots of *T. wilfordii*. In addition, the need for improved techniques for the analysis of various plant toxins which might be present in grazing foodstocks or herbal teas continues. The most important of these toxins are the pyrrolizidine alkaloids, and Molyneux and colleagues have described an improved reverse-phase, ion-pair HPLC method using a phosphate buffer [164] for the separation and identification of 32 alkaloids of this class.

Capillary electrophoresis (CE) has undergone some dynamic changes in the recent past, particularly with respect to the use of higher applied field strengths [165] and the use of borate buffers to improve resolution [166]. Now McGhie and Markham [167] have described the electrophoretic mobilities of 33 closely related flavonoid glycosides through CE and the separation of 20 of these in a single run. Other recent applications have been for the separation and quantitation of the constituents of *Eleutherococcus senticosus* [168] and for the quaternary isoquinoline alkaloids in the rhizomes of various commercial samples of *Coptis* species [169], which are used in traditional Chinese medicine.

The advances made through the introduction of thermospray liquid chromatography mass spectrometry were reviewed by Hostettmann and colleagues [170] and a summary given of the numerous diverse applications in the analysis of almost every class of natural product. The technique is extremely powerful, offering a UV trace of constituents absorbing at a given wavelength, a total ion current trace, and a mass spectrum and daughter ion spectrum of each region of the chromatogram. For individual compounds of interest, selective ion monitoring can also be applied. As discussed elsewhere in this review, electrospray techniques coupled with natural product data bases may overcome some of the disadvantages of thermospray technology. A detailed comparison of various LC–MS interfaces, particularly as they apply to the analysis of alkaloids has been made by Verpoorte and Niessen [171]. Finally, it is worth mentioning that mass spectrometry can now be applied for the molecular weight estimation of certain lower molecular weight enzymes. Thus, Schreier and coworkers have described the very effective use of matrix-assisted laser desorption/ionization mass spectrometry and electrospray ionization mass spectrometry for the differentiation of soybean lipoxygenase isoenzymes on comparison with gel permeation chromatography [172]. Molecular masses were in the range 93 000–96 500.

STRUCTURE ELUCIDATION

Most of the papers which appear in the principal journals of natural product chemistry are concerned, in whole or in part, with the structure elucidation of a natural product, or some derivatives thereof. These products are isolated from a diversity of sources and possess a very broad array of structures. Typically in a year, several new natural product skeleta are isolated and characterized from these various sources. Many of the isolation procedures result in a known compound and this happens in both phytochemical work and in efforts involving bioactivity-directed fractionation. Numerous studies are made each year analysing crude natural products for their claimed active ingredients either for the purposes of identification (evaluation for adulterants) or for standardization of the active ingredient(s). In all of these cases, there is a profound need to characterize a compound or series of compounds. The compound may be isolated or it may be retained in the matrix of the extract and the complete extract or a selected fraction thereof characterized. In all of these instances, a structure elucidation or verification has occurred at some point.

The basic techniques for the structure elucidation of natural products have changed very little in the past ten or fifteen years, they are still IR, UV, proton and carbon NMR spectroscopy, ms, optical rotation, and circular dichroism. What has changed, principally as a result of the computer software revolution, is the level of automation that has been brought to instrumentation, the sophistication of the experiments that can be performed, and the rate at which data can be integrated into a coherent structure. One standard has changed, namely that proton and carbon-13 assignments are now proved and not 'guessed at' following a series of chemical shift calculations. This is a very significant philosophical change, to which we have been a contributor in recent years [173–175], for it means that chemical shifts for a given biologically active compound can be used with far greater assuredness in determining the structures of related compounds, in evaluating *in vitro* the site of binding/action of the compound with an enzyme and the location of incorporation of a stable isotope-labelled biosynthetic precursor into the molecule. Thus, it would be unusual now to see a new structure being determined where the resonances were not completely (or almost completely) assigned, unambiguously, through a series of one- and two-dimensional NMR experiments.

The range of NMR techniques that are available to the natural product chemist interested in the structure elucidation of new metabolites is almost bewildering. Several books have appeared discussing the use of techniques [176–178], one of them of special interest to natural product chemists [179]. Many of the techniques have significant drawbacks in terms of resolution required, time, sample size, computer capabilities, etc. and these aspects have been discussed [180, 181]. Nonetheless, the following list of techniques, mostly described or extensively applied in the past ten years represents those which are most useful in structure elucidation and unambiguous assignment: COSY [182, 183], DQF-COSY [184,185], HOHAHA [186–189], NOESY [190], ROESY [191–193], HETCOR [194–197], HMQC [198], HMBC [199, 200], FLOCK [201, 202], Selective INEPT [203], COLOC [204], and INADEQUATE [205, 206].

What has changed dramatically in the recent past is the strategies employed for the elucidation of natural product structures, both in terms of gross structure, as well as the subtleties of relative and absolute stereochemistry, and a recent chapter [207] has highlighted the importance of some of the key NMR techniques and how they can be applied. The strategies that are selected are also frequently time-sensitive, which means, if high-field instrument time is charged, as it often is in academic institutions, that one needs to consider the best way in terms of available techniques, sample size, time and cost to gain a particular result. For example, very similar data can often be obtained from the long-range HETCOR, FLOCK, selective INEPT and HMBC spectra. How-

ever, if only a limited number of ^{1}H–^{13}C correlations are needed and there is adequate sample, the best results are probably obtained with a series of selective INEPT spectra rather than say an HMBC spectrum. We have discussed elsewhere some of the strategies, involving a combination of one- and two-dimensional NMR techniques, which we have successfully used for the unambiguous assignment of the proton and carbon spectra of various groups of triterpene and steroidal saponins [208–211].

The advent of these sophisticated NMR techniques, coupled with enhanced biosynthetic knowledge, improved isolation techniques which afford purer products, and a hands-on situation between the isolation chemist and the spectroscopic equipment has resulted in a number of structures of compounds of various classes being revised [212] or identities established [213], and in the determination of increasingly sophisticated structures of which maitotoxin is particularly noteworthy [214].

McLaughlin has established many of the characteristic NMR spectral parameters for the determination of the structures of the cytotoxic acetogenins of the Annonaceae [215,216], which also possess antimalarial, pesticidal and antimicrobial activity. These compounds appear to act as inhibitors of NADH: ubiquinone oxidoreductase activity in complex I and thereby inhibit glutamate-dependant mitchondrial respiration. Because of the number of stereocentres represented by the many tetrahydrofuran rings, as well as the many secondary alcohol units on the chain, complete structure determination is an interesting challenge. For example, the advanced Mosher ester methodology [217] was used to determine many of the secondary carbinol centers. This technology has been important in recent years for the absolute determination of isolated alcohol units [218,219]. The technique relies upon the differences between the ^{1}H NMR chemical shifts of the *S*-and *R*-methoxy-(trifluoromethyl)-phenylacetate ester derivatives on either side of the chiral center. When $\Delta\delta_H(\delta S-\delta R)$ is positive the center has the *R* configuration and *S* when the same difference has a negative sign. Needless to say, the technique requires unambiguous assignments of the protons on either side of the stereogenic center.

Some other examples also give evidence to the power of modern structure elucidation techniques. A group of poly-galcic acid 3,28-*O*-bisglycosides possessing six or seven monosaccharide units, the masonosides, have recently been characterized from the corms of *Crocosmia masoniorum* in the Iridaceae [220]. For masonoside A, a parent ion at *m/z* 1969 representing $[M + Na]^{+}$ was observed and a ^{1}H NMR spectrum with eight anomeric carbons. Various hydrolytic techniques coupled with mass and NMR techniques led to a complete structure determination.

Koshimura and associates have described a new technique for determining the binding site between lignin and the glucuronic acid residue of xylan [221] using an acid-conjugated DDQ oxidation of the benzyl esters. The analysis of a water-soluble lignin–carbohydrate complex from beech wood by carbon-13 NMR permitted an estimate to be made of 1.6 ester bonds between the lignin and the glucuronic acid residue of glucuronoxylan in the complex.

Because of the need for a substantial amount of sample for the analysis, the INADEQUATE experiment [222], which directly examines carbon–carbon connectivity, has been relatively little used for the structure elucidation of new natural products. O'Mathúna and Doskotch [223] recently described the use of this technique for the structure elucidation of amoenolide A. All but three of the carbon–carbon bonds were determined directly for this labdane diterpene.

The TOCSY experiment [224], as a one-dimensional technique [225], has also not been very widely used, although this powerful technique does have the ability, through varying the mixing time, of tracing correlations through extended matrices, and has been used for oligosaccharides [226] and tomatidine [227], and more recently for the complete assignment of the protons and carbons of several *Solanum* glycoalkaloids [228].

Standardization of medicinal plant samples has become a growing concern in many countries in the past few years, particularly in situations where regulations regarding either safety or efficacy are not applied prior to the point of sale to the consumer. While this is a vast area of discussion and debate with respect to new regulations in the U.S.A. at the present time [229], there remains a need for reliable, simple, quantitative procedures. Following earlier work on the qualitative analysis of the principles of *Croton lechleri* [230] by ^{1}H NMR, Phillipson and coworkers have now developed quantitative HPLC and ^{1}H NMR techniques for the analysis of paeoniflorin in red peony roots (*Paeonia lactiflora* and *P. veitchii*), using the aromatic proton signals as markers [231]. A good correlation was observed between the HPLC and NMR data in the analysis of seventeen samples. Significant differences in the levels of paeoniflorin (range of 0.1–4.5%) were found, indicating that some samples were not of adequate quality.

Of course, NMR spectroscopy is not the only physical science where substantial progress has been made resulting in a tremendous impact on the way in which natural product chemistry is conducted. Many strides have also been made in mass spectrometry, particularly in terms of quadrupole desk-top instruments which, linked to gas chromatography systems, provide a powerful, moderately priced, analytical tool.

With highly polar materials, highly reactive cations, or with materials which have sensitive functional groups, obtaining a molecular ion frequently provides a significant challenge as an integral part of the structure elucidation process. Thus, for example, although fast atom bombardment mass spectrometry of anthocyanins was reported some time ago [232], the technique does not work for the corresponding aglycones, the anthocyanidins. Derivatization and GC–MS analysis helps somewhat, but is limited by the large sample size required [233]. Plasma desorption mass spectrometry (PD–MS), which uses a ^{252}Cf ion source [234] has recently been

applied by Nicholson and colleagues to the analysis of anthocyanins and anthocyanidins, both pure and in a plant extract matrix at the nanogram level without any derivatization [235]. In their studies of the *seco*-glycosides of oleanolic acid from *Beta vulgaris*, Connolly and coworkers also resorted to PD-MS to obtain the molecular ion of a derivative when positive ion FAB afforded only a quasi molecular ion species [236].

Yet another analytical technique which has evolved rapidly in the past few years has been protein sequencing, and the impact of this technique has been felt in many areas of plant chemistry. Mino and coworkers has recently been applying protein sequence determination in the area of chemotaxonomy to the genus *Datura* [237–239]. Using ferredoxin, an iron–sulphur transfer protein, as a marker, Mino has examined two varieties of *D. stramonium* [237], two *Datura* species (*D. metel* and *D innoxia*) [238], and *D. arborea*. For *D. arborea* only three amino acids were different from the Ds-ferredoxin and four from the Dm-Fd; three differences were also observed between the Ds and Dm-ferredoxins, supporting the idea of different sections of the genus *Datura* rather than different species for the arboreal forms.

BIOLOGICAL TESTING

Whether the target is a biological (e.g. insecticide, herbicide) or a therapeutic one, no area of natural product discovery has changed as much in the past 10 years as biological evaluation [10, 240, 241]. A book [242] and several recent conferences attest to the high level of interest in the biological evaluation of natural product samples, particularly in highly automated, high volume screens. The book brings together a wide range of bioassays, including those for antifungal [242a], antibacterial and antiviral [242b], anticancer [242c], antimalarial [242d], molluscicidal and shistosomicidal [242e], plant-insect interactions [242f], immunomodulators [242g], hepatotoxic [242h] and platelet aggregation [242i].

It is not so very long ago that a pharmaceutical company would boast that in the past year it had completed the biological evaluation of 15000 samples in two or three assay systems. These were probably *in vivo*, or possibly cell-based assays and the compounds were probably 99% derived from synthetic or antimicrobial sources. The advent of automated high-throughput screening has dramatically changed three aspects of this paradigm: (i) the rate at which samples are evaluated, (ii) the biological specificity being measured, and (iii) the length of time that an assay is actually in operation. These changes also occurred at a time when the use of animals in research came under substantial scrutiny and ethical evaluation for the appropriateness of the research, particularly when animals were placed under extended duress prior to death or sacrifice. Organ-based assays [243], have now been replaced as the front-line primary screen, but often retain significance as secondary screens to assist in prioritization of active extracts or compounds. They represent an essential connection between the high technology of the primary screens and the realities of pharmacological effectiveness.

The very substantial changes that have occurred in the understanding of fundamental biological processes related to a number of major disease states, coupled with the enhanced availability of numerous receptors, enzymes, genetic switches and cell systems has allowed, in some instances, the use of these as automated primary bioassay systems. These systems permit the screening of tens of thousands of samples in a week, and thus whole libraries of samples (100–150 K different specimens) may be screened within a few weeks. Thus, the numerous pharmaceutical companies, who are invested in random screening programs as the first step in their discovery programs, must focus on maintaining and diversifying their chemical bank of samples to be screened. One way to do this is to collect diverse plant samples. Since each sample may comprise 30–40 assayable compounds (others will be present at too low a level), a few thousand or so plants will have a dramatic impact on the chemical diversity which can be evaluated rapidly in a given bioassay.

As a result, many automated bioassays have a very limited lifetime of only a few weeks or months, enough to screen the whole 'bank' of chemodiversity and evaluate the results, before a new assay, seeking a specifically acting compound in a different selective assay is put in place. Thus, as a part of a new strategy for establishing chemical banks of great diversity, many pharmaceutical companies have returned to the global collection of plants, not just for now, but as a resource for their discovery efforts into the next century.

Some companies have also based their plant collection programs on either an ecological or an ethnomedical approach. In this instance, the need for automated assays is substantially reduced and indeed the tests may be *in vivo* assays, depending on the activity or the pathophysiology of the disease. In any event, the sample size required is somewhat larger, and such assays may provide valuable information regarding overall efficacy and toxicity which is not obtainable through automated screening.

The pace at which the screening of samples can be achieved has also been dramatically enhanced by the introduction of radioligand binding assays. Their reliability, simplicity and sensitivity has meant that many whole animal or organ assays can be eliminated as initial screens. Typically, a specific binding of $\geqq 80\%$ is needed for high volume screening, and should accurately reflect the interaction between the drug and the receptor, and not be a non-specific binding interaction, for it is important that the assay be run at equilibrium. Sweetnam and colleagues [244] have described the importance of receptor binding assays in the drug discovery process, the factors involved in establishing high-volume screens and how comparative data from 38 different primary assays can be presented for review.

The concept of a 'hit rate' is a very difficult issue in many pharmaceutical companies, depending on how management, chemists and the biological screening group view

their respective roles. If a hit rate is set too high, a plethora of actives will result which will have to go through a secondary biological or intellectual screening process. If the rate is set too low, insufficient lead structures for isolation and medicinal chemistry development may be generated. However, what does evolve will probably be the most potent, and therefore in the view of many, the most highly desirable, as a potential candidate for development, the ultimate test of a screening programme. Thus, some companies in reviewing a library of 50,000 samples may set a rate of 1% actives (500 samples) for a screen, whereas another may decide that a 0.01% rate (five samples active) is more appropriate.

Another phenomenon that has occurred as a result of the revolution underway in the area of biological screening is that 'old', sometimes very widely distributed, compounds are displaying activities, sometimes selectively, against certain enzymes and receptors. One of the major groups of compounds where numerous new biological activities have been found in recent years are the flavonoids [42, 245].

The flavonoids, for example, have been shown to be able to inhibit numerous diverse enzymes, including various hydrolases, cyclooxygenase, alkaline phosphatase, cAMP phosphodiesterases, several ATPases, lyases, hydroxylases, transferases, oxidoreductases and kinases [245]. Several of them are potent estrogenic materials, are antimutagenic, and possess anticancer activity. Although widely occurring and an integral part of the dietary intake of all herbivorous species and a constituent of many ancient remedies, few flavonoids have been well investigated biologically or afforded clinical trials.

When the mechanism of action of compounds is investigated for the ability to interfere with certain enzyme processes, the importance of a compound may be dramatically altered. There are two examples of such instances occurring in the cancer field, one of these, taxol, has been partially discussed, the other is camptothecin. Both compounds were isolated as a result of bioactivity-directed fractionation for KB activity, camptothecin from *Camptotheca acuminata* [246] and taxol from *Taxus brevifolia* [86]. Subsequently, they were each evaluated in several *in vivo* models [247]. Sodium camptothecin, a substantially less active derivative, was given a clinical trial, following which interest waned due to the observed gastrointestinal and other toxicities [248], although clinical trials with the parent alkaloid continued in China [249]. When the mechanism of action of these compounds was investigated, each was found to be unique: camptothecin was an inhibitor of topoisomerase I [250, 251] and taxol promoted tubulin polymerization [71]. It was only then that interest in these compounds was revived. Several analogs of camptothecin are now in clinical trial or in advanced stages of development [252, 253], and the synthesis of taxol analogs is a burgeoning area [75, 77, 81, 82, 254–256].

This reflects a major change in decision-making strategy with respect to which compounds to pursue for development purposes. Raw potency (there are numerous compounds more cytotoxic than camptothecin) or even structural novelty (neither taxol nor camptothecin were ever patented) may no longer be the critical issues. An additional example arose in our laboratories recently where a very widely distributed steroid, betulinic acid, was found to possess highly selective activity against human melanoma *in vitro* and *in vivo*. The novel mechanism of action, which appears to be related to apoptosis, suggests that this compound or a derivative thereof, may also hold promise as a therapeutic agent [257]. This work arose from a program sponsored by the National Cancer Institute as a part of two major new initiatives underway.

The history of natural product drug discovery at the National Cancer Institute has been well documented [258]. The disclosure regarding the unique biological activity of camptothecin and taxol led to a reconsideration of natural products as sources not only of anticancer agents, but also of anti-HIV agents. Two programmes were developed, one an intramural program focussing on anticancer [109, 111] and anti-HIV [110, 112] agents, and a second the National Cancer Natural Products Drug Discovery Grant program [259, 260]. The former program contractually relies for its source materials on plant and marine sample collectors who ship materials to NCI where it is processed, and the extracts submitted to a panel of approximately 60 human cancer cell lines in nine therapeutic areas (leukaemia, non-small-cell lung, colon, brain, melanoma, ovarian, renal, prostate and breast). Rather than seeking a very potent generally cytotoxic agent, which was an earlier criteria, selectivity against certain groups of cell lines is sought, at which point the extract is prioritized for fractionation in that cell-line and the selectively active principle evaluated in an *in vivo* animal model which reflects the observed *in vitro* selectivity. In the anti-HIV discovery program, a whole cell assay is being used, looking for agents which will prevent the cytopathologic effects of the infecting virus in the host cell.

The NCNPDDG program has a requirement of an industrial partner, which, in our experience during the past 5 years, has brought to the table a very important focus on critical decision-making. Seven of these grant awards were made; some in the plant area and some in the marine area. Each group had a different biological approach to their drug discovery effort, including inhibition of DNA repair, DNA damaging agents, protein kinase C inhibition, etc. These various programs have been described in a symposium volume [259]. The program devised by our consortium group has also been described in some detail [7, 8, 11, 12, 14] and will only be outlined here. This program, a collaboration between groups at the University of Illinois at Chicago, Research Triangle Institute and Glaxo Research and Development in the U.K. focused on plants which were collected based on their ethnomedical use and their endemicity from over thirty different countries. The extracts were then evaluated in about 28 different assays, some whole cell-based, some enzyme-based and some looking at specific marker events in oncogenesis. With over 2500 plant samples collected, an important aspect of the program has been

the ability to catalogue all of the botanical, chemical and biological data in a form which is readily accessible to all parties, and from which meaningful reports can be generated for analysis of the data by the whole group at regular intervals. Once again, the biological focus is on selectivity of action and, as indicated with the example of betulinic acid given above, only secondarily on novelty of structure.

Other approaches to the discovery of anticancer agents have been described which also reflect changing strategies away from the former use of a single cytotoxicity test. We have described how very crude DNA can be used as a tool for the HPLC analysis of extracts looking for agents in complex mixtures which would bind, non-covalently, to DNA [261]. We have also described the use of a genetically engineered human cancer cell line which is specifically resistant to certain anticancer agents. Thus any compound which shows cytotoxicity in this cell line and the parent cell line may have the ability to overcome the drug resistance. Several such types of compound have been discovered in this manner [262–266]. Another approach is to look for agents that will inhibit DNA repair [267], i.e. after a primary agent has inhibited DNA activity through scission, a second agent inhibits the repair mechanisms which operate to restore the original activity.

McLaughlin and coworkers have focussed attention on two simple-to-perform tests [268, 269], the brine shrimp assay [270] and the potato disc assay [271] as alternative prescreens for antitumor activity. These screens are quite well correlated with cytotoxic activity [272], have minimal equipment requirements and have effectively led to several interesting groups of active compounds [273]. The potato disc assay (induction of crown gall tumours by Ti plasmids in *Agrobacterium tumefaciens*) has been used for the screening of several rare, non-antimicrobial natural products [274]. Of these, monorden, diplodiol and chaetoglobosin K were shown to be of potential interest.

While cancer and AIDS have attracted a lot of research attention in the U.S.A. because of the available government funding for discovery programmes, there are numerous other biological areas which have been investigated for the provision of lead compounds for drugs or biological agents. Some of the biological activities evaluated for plant-derived natural products and the type of compound isolated as a result of those researches during 1994 as cited in the *Journal of Natural Products and Phytochemistry*, exclusive of cytotoxic or anticancer [275–289] and anti-HIV [290–294] compounds derived from higher plants, are listed in Table 1 [295–373]. This is not a comprehensive list from these journals and

Table 1. Some citations regarding biological activity from the *Journal of Natural Products* and *Phytochemistry*

Biological assay system	Type of active compound	Reference
Acaricidal activity	Chromenes	295
Adenylate cyclase inhibition	Manoyl oxide derivatives	296
ACE inhibition	Huperzine derivatives	297
Anti-dust mite activity	Lanostane triterpenes	298
Anti-*Encephalomyocarditis* activity	Flavonoid	284
Antifungal activity	Methylthiopropenoic acid amide sulphones	299
	Phenalenone derivatives	300
	Prenylated isoflavone	301
	Prenylated flavanones	302
	Xanthones	303
	Rosmarinic acid	304
Anti-histamine release activity	6-*O*-Galloyl glucose	305
Antihypertensive	7-oxo-10α-cucurbitadienol	306
Anti-inflammatory activity	Naphthalene-isoquinoline alkaloids	307
Antimalarial activity	Abietane diterpenes	308
Antimicrobial activity	Anthraquinone	285
	Sesquiterpenes	309
	Sesquiterpenes, diterpenes	310
	Trichorabdane diterpenes	311
	Isoflavans	312
Antimutagenic activity	Gallocatechin	313
Anti-oxidant activity	Cinnamiphilin (lignan)	314
	Depsides and depsidones	315
	Xanthones	316
Antirhinovirus activity	Sesquiterpene hydrocarbons	317
Anti-tumour promoting activity	Furanonaphthoquinones	318
	Steroidal saponins	319
Anti-VSV activity	Abietane diterpenes	320
Ant repellant	Sesquiterpene	321
Bacillus subtilis	Dihydrochalcones	322
	Triterpene acids	323

Continued Overleaf

Table 1 *Continued*

Biological assay system	Type of active compound	Reference
Botrytis cinerea inhibition	Sesquiterpene hydrocarbons	324
Ca^{2+}-ATPase inhibition	Hydroxystilbene glucosides	325
Cell adhesion inhibition	Cucurbitacins	326
Cerebral blood flow enhancement	Dehydroevodiamine	327
Cytoprotective activity	Dehydroleucodine	328
DNA repair in yeast inhibition	Oxo-aporphine alkaloids	329,330
Human lymphocyte proliferation inhibition	Patuletin rhamnoside derivatives	331
Hypolipidemic activity	Phenolic diarylheptanoids	332
Immunosuppressive activity	Cyclic monoterpene	333
Insect antifeedant	Dihydro-isocoumarin glucoside	334
	Limonoid	335
Insecticidal activity	Chromenes	295
	Furanocoumarins	336
Insect growth inhibition	Lignans	337
	Neolignans	338
Interleukin-1 inhibition	Diterpene quinoids	339
Larval growth inhibition	Steroidal saccharide esters	340
Leishmanicidal	Flavans	341
	Aporphine alkaloids	342
β-Lactamase inhibition	Anacardic acid	343
5-Lipoxygenase inhibition	Diphenyl ether	344
15-Lipoxygenase inhibition	Anacardic acids, cardols	345
Micrococcus luteus	Dihydrochalcones	322
	Triterpene acids	323
Molluscicidal activity	Triterpene saponins	346
Monoamine oxidase inhibition	γ-Pyrone derivative and xanthones	347
Moth defence secretion	Cyanoglucoside	348
Mushroom tyrosinase inhibition	Anacardic acids, cardols	349
Nematocidal activity	Diphenylheptanoids	350
	Chromenes	295
Neurotensin binding inhibition	Cyclic peptide	351
Nicotinic/muscarinic acetylcholine receptor binding	Quinolizidine alkaloids	352
Oral Antimicrobial	Isoflavonoids	353
Oviposition inhibitors	Polyphenolic cinnamoyl anthranilic acids	354
Oviposition stimulant	Flavanol triglycoside	355
Plant cytotoxicity	Purine riboside	356
Plant growth inhibition	Phenethyl α-pyrones	357
	Sesquiterpene lactones	358
	3-Hydroxy-β-ionone	359
Platelet aggregation inhibition	Anthraquinones	360
	Aporphines, furoquinolines, haplopine (?)	361
	Aristolochic acids	283
	Benzophenanthridines, furoquinolines	362
	Cinnamiphilin (lignan)	314
	Dihydrostilbenes	363
Positive inotropic activity	Steroidal saponins	364
Propionibacterium acnes	Anacardic acids, totarol, δ-cadinene, β-caryophyllene	365
Protein kinase inhibition	Acridines, aza-acridines	366
Protein kinase C inhibition	Catechins	367
	Polyphenolic cinnamoyl disaccharides	368
Retinoid receptor X activator	*cis*-Retinoic acid derivative	369
Root growth inhibtion	Brassinosteroids	370
Seed germination inhibition	Phenyl acetylene	300
Trypanosoma cruzi inhibition	Aporphine alkaloids	342
Vasodilation activity	Furofuranolignans	371
Vasopressin receptor binding inhibition	Sesquiterpene alcohol	372
Vinblastine binding inhibition	Iboga alkaloids	373

clearly does not represent the diversity of biological evaluation reported in the broader primary literature. However, these 55 or so activities, each of which reflects a series of active natural metabolites, attests to the diverse biological potential of plant-derived products, and to numerous novel metabolities with activity that may serve as potential lead candidates for development.

NEW SOURCES OF NATURAL PRODUCTS

As well as a major emphasis on searching for plants from the areas of greatest biodiversity, there have also been two other directions which investigators have taken to enhancing chemodiversity. One is to seek plants from unusual regions of the world, the second is to examine previously poorly studied sources. In the former category, some aspects of the extensive research performed on the plants of New Caledonia by French investigative teams in the past 30 years have been published [374]. Another area of the world whose flora is being examined is the Antarctic. As part of a project aimed at examining changes in the UV-B and ozone levels in the Antarctic, the major flavonoid constituents of the grass *Deschampsia antarctica* were studied [375], and the quercetin glycosides from three species of *Ranunculus* growing in the Kerguelen Islands have also been studied [376]. These ecological studies may prove to be significant as we seek ways to monitor the changes in our plant environment caused by environmental degradation.

Other new sources which have been studied include: the cultured chrysophyte, *Poterioochromonas malhamensis*, which afforded a novel protein tyrosine kinase inhibitor [377], and the Magpie moth, *Abraxas grossulariata*, once an abundant species in Britain, but now almost disappeared, which afforded the cyanoglucoside sarmentosin [378], previously only isolated from a Chinese *Sedum*. Cytotoxic agents have been isolated from the moss *Polytrichum pallidisetum* [379], and Asakawa has continued his interesting studies on the liverworts [380, and Refs therein], which continue to yield structurally and biologically interesting metabolites.

One new source of fungal products which has been studied recently are the coprophilus fungi which have provided a number of new antifungal agents [381], the latest of which is apiosporamide, a metabolite of very mixed biosynthetic origin from *Apiospora montagnei* [382].

In the section on structure elucidation, mention was made of the analysis of samples by physical means for the purposes of identification. One of the strategies that has changed recently in terms of standardization of plant materials, extracts, and specially prepared mixtures, is the use of their biological activity as a measure of their quality, a facet which appropriately has important regulatory ramifications [383]. Such a change has occurred for several different reasons, including the availability of routine *in vitro* bioassays and the recognition that defined biological activity, although sometimes difficult to measure because of interfering substances, can be a valuable criteria in assessing the worth of a particular plant sample. In Thailand, for instance, there has been renewed effort in recent years to establish assays and standards for several of the major plant crops that are exported for their medicinal, biological or condiment properties, with the emphasis being increasingly placed on *in situ* analysis as a way to improve the price of the goods on the open market, and thereby the return to the farmer.

Synthesis

The complexity of the structures of most natural products, even those with few or no chiral centers, precludes their commercial synthesis. Yet in the course of a year numerous natural products, some quite simple, others like taxol very challenging, are synthesized, and many organic chemists devote substantial effort to the intellectual challenge of effectively producing these molecules with high stereoselectivity and in high efficiency, often devising exceptionally creative reagents to address complex molecular manoeuvrings [384, 385]. Cornforth, in an interesting presentation has discussed some of the changing reasons for conducting synthetic work [386].

One rationale offered for this tremendous effort, which will not be debated here, is that these schemes provide routes to the development of analogs for biological evaluation. It is certainly the case that many innovative, elegant and quite efficient syntheses of complex natural products have been devised, indeed almost every issue of the *Journal of the American Chemical Society* or the *Journal of Organic Chemistry* or *Tetrahedron* contains examples of such syntheses. The former rationale of total synthesis was that it was needed to justify the assignment of a natural product structure and in our own experience some of our structures have been verified by synthesis (jacaranone, microminutin, aristolindiquinone, nirurine, etc.), while others (prionitin) have not. There are indeed a number of instances each year where a structure is corrected through synthesis, but for the most part the structure is well-established before synthetic work is initiated. This is crucial because the two primary strategies for natural product synthesis, retrospective analysis [385] and biomimetic synthesis, necessitate a well-characterized structure.

A multitude of creative talent has contributed to the synthesis of natural products in the past few years and in certain cases (e.g. camptothecin, ellipticine, cephalotaxine, retronecine) a very substantial number of syntheses have been reported. In other instances (e.g. ergot nucleus, vincamine, morphine, yohimbine, taxol) emphasis has been placed on generating analogs systematically related to the natural product.

An alternative approach to the synthesis of natural products and their relatives is semisynthesis, which can take on any one of a number of forms. For example, it may be directed at the natural product per se, at simple derivatives of the natural product using the parent compound as the starting material, at derivatives of the natural product beginning with another natural product, at substantially modified natural products using the skeleton as a template, or at pharmacophoric units of the

bioactive molecular species. Each of these approaches has special circumstantial merit often limited by the complexity or availability of the compound. The ability to effectively introduce or maintain chirality in reaction processes has been the principal focus of organic synthesis, and is now refined to the use of chiral auxiliaries [387], catalytic asymmetric synthesis [388, 389] and the use of chiral starting materials, such as sugars [390].

As more and more mechanisms of natural products are being investigated at the molecular level in binding or displacement assays, there is an increasing need for the compounds to be made available in labelled, often tritiated, form; the daphnane ester resiniferatoxin [391] and taxol [392] are two recent examples. The standard approaches for such labeling of an aromatic compound involves either catalytic tritiation of a halide derivative or an olefin of the natural product. A new strategy for tritiuim labeling has been described [393] which can be applied to underivatized compounds. It was used initially on ibogaine, a compound of substantial interest as a potential anti-addiction treatment for cocaine abuse [394]. The procedure involves the low-temperature treatment of the lithium anion of ibogaine with potassium *tert*-butoxide followed by tritium gas to afford a 1: 1 mixture of the 11- and 12-tritiated species which could be used for metabolism, pharmacokinetic and binding studies.

The rise in the abuse of cocaine has also led to other needs for analogues as potential anti-addictive agents and as imaging agents for various neurodegenerative disorders [395]. Neumeyer and coworkers [396] have also developed radioligand probes of cocaine for PET and SPECT imaging of cocaine receptors using iodine as the ligand. Another new approach to the development of anti-addictive strategies for cocaine is the formation of monoclonal antibodies which can accelerate the hydrolysis of cocaine *in vivo* thereby making it ineffective as a stimulant [397].

The synthesis of precursors, particularly those to be used for stable isotope biosynthetic experiments has also been an area that has developed rapidly in the past few years. One recent example in the case of podophyllotoxin biosynthesis is the stereospecific synthesis of (7′α-$^2H_1$)-yatein, an advanced precursor on the route to podophyllotoxin [398], in which only closure of ring B is required.

BIOSYNTHESIS

Biosynthetic studies have also changed dramatically in the past few years. The principal changes have involved the use of stable isotopes, particularly carbon-13, deuterium, oxygen-18 and nitrogen-15 [399], culture of plant cells [400], advances in the techniques for the isolation of enzymes in the biosynthetic pathway [40, 401], and the cloning and expression of the genes for those enzymes [402]. In the case of the erythromycin antibiotics, the gene for the expression of the antibiotic has been isolated [403], permitting the more precise manipulation of the system with respect to precursor relationships and therefore the production of new metabolites through targeted gene disruption [404, 405].

In the biosynthesis of plant secondary metabolites, with many of the precursor relationships established through earlier radiolabeled feeding experiments, several of the major pathways are now being investigated in greater detail in tissue culture, in cell-free systems or at the enzyme level. One of the crucial issues to be resolved though is the relationship between the metabolites of the whole plant and the callus or cell-free systems, because frequently these are quite different [401]. For example, in a study of pentacyclic triterpenoid biosynthesis, Ayabe and coworkers [406] found that the regenerated plants and the wild plants of *Taraxacum officinale* contained no triterpene acids, although oleanolic and ursolic acids were major constituents of the undifferentiated callus cells. Amazingly, cell cultures of *Papaver somniferum* or *P. bracteatum* do not produce morphinan alkaloids [407], but rather sanguinarine; an opportunity, not a problem, as related below. Earlier work with *Cinchona* cell cultures produced very low levels of quinine and quinidine, anthraquinone biosynthesis becoming dominant [408, 409]. Some successes have been achieved however, and the production of berberine from *Coptis japonica* in yields of 7 g l^{-1} after screening of cell lines and optimization of culture conditions, make this the highest yield of an isolated product from plant cell cultures.

An example of the use of deuterium in examining the stereospecificity of reductions and eliminations in biosynthetic pathways has recently been reported by Moore and Floss in their studies of the formation of cyclohexane carboxylic acid from shikimate in *Streptomyces collinus* [410] and *Allicyclobacillus acidocaldarius* [411]. Using [2,6,10,10-$^2H_4$]-chorismate they were able to show that the path to cyclohexane carboxylic acid branches at either the stage of shikimate or shikimate 3-phosphate and not chorismate.

Cytokinins are known to regulate both plant growth and development and also the production of secondary metabolites, including indole alkaloids [412]. Now it has been shown that 6-benzylaminopurine (BAP) promotes the biosynthesis of berberine in *Thalictrum minus* cells through the induction of norcoclaurine-*O*-methyltransferase [413], a key intermediate in the pathway for the formation of coclaurine. Tetrahydroberberine oxidase was also enhanced by the addition of BAP, whereas five other enzymes in the pathway were unaffected.

The methylenedioxy group is regarded as forming between a phenol and an oxidized methoxy group, and an enzyme catalyzing such a reaction was first isolated by Zenk's group [414]. Many berberine alkaloids and the skeleta produced from them also contain such a group, and Rueffer and Zenk [415] have reported on the isolation of canadine synthase from *Thalictrum tuberosum* cell cultures which converts (*S*)-, but not (*R*)-tetrahydrocolumbamine to canadine. The enzyme is microsomal bound, cytochrome P-450 dependant and is widely distributed in the Berberidaceae and the Ranunculaceae.

A major change in the study of biosynthetic pathways in tissue culture in the past few years has been the addition of fungal elicitors in order to enhance the pro-

duction of key secondary metabolites [416–418], including alkaloids [419,420]. Many groups have now used this technique in order to study alkaloid biosynthetic pathways and for the production of alkaloids. Zenk and coworkers, for example, have used this technique very successfully [421] to elucidate all of the eight enzymes in the pathway from (*S*)-reticuline to sanguinarine using an elicited culture of *Eschscholtzia californica* [422]. It was the addition of fungal cell wall constituents to the cultures of *Papaver somniferum* which led to enhanced sanguinarine biosynthesis and to a production process using immobilized cells [423].

Macarpine contains two additional methoxy groups, at positions 10- and 12- compared with sanguinarine. These alkaloids are each produced by the action of a dihydrobenzophenanthridine oxidase on the corresponding dihydro derivatives. Thus, dihydrosanguinarine is projected to be a precursor of dihydromacarpine, which is then oxidized. The four enzymes in this pathway which oxidize dihydrosanguinarine at C-10, methylate the phenolic group, hydroxylate dihydrochelirubine at C-12 and then methylate the phenolic group have now been isolated by Zenk's group [424,425]. This twelve enzyme sequence represents that longest biosynthetic pathway of a secondary metabolite in a plant which has been deduced at the enzyme level.

Mahady and Beecher have also studied benzophenanthridine biosynthesis in *Sanguinaria canadensis* cell cultures treated with either an elicitor derived from *Penicillium expansum* or the calcium ionophore A23187 [426]. The results demonstrated that an external source of Ca^{2+}-ions is required for elicitor-induced benzophenanthridine alkaloid formation, and that calcium may have a role in a signal transduction system which regulates this biosynthetic pathway. The inorganic aspects of natural products biochemistry are thus revealed.

Tissue cultures for the formation of medicinally important alkaloids continue to be established. One recent example is for camptothecin and 9-methoxycamptothecin using an indigenous tree *Nothapodytes foetida* [427]; alkaloid levels were substantially below those of the mature tree. The secondary metabolism of *Artemisia annua* tissue cultures has been studied for several years, but with little success as far as producing the desired artemisinin. Recent studies by Brown have highlighted this problem showing that while the undifferentiated callus produced no artemisinin, the differentiated shoots had a terpenoid profile similar to that of the intact plant [428].

Tissue culture work frequently requires the screening of substantial numbers of plant cell cultures for which rapid and reproducible assays are needed for the compound in question. Loyola–Vargas and coworkers have described combined TLC-densitometric techniques for the quantification of serpentine and ajmalicine in plant cell cultures of *Catharanthus roseus* and of hyoscyamine and scopolamine in *Datura stramonium* [429]. The detection limit for serpentine was 20 ng and 500 ng for the other alkaloids. Radio-immunoassay analysis for scopolamine, as well as polyclonal antibodies, have also been used, but recently [430] Sawada and coworkers described a monoclonal IgG1(k) antibody with high affinity for methylscopolamine and which had weak cross-reactivity with atropine, and hydroxyhyoscyamine derivatives. The sensitivity allowed detection of 20 pg per assay.

Tropane alkaloid biosynthesis has indeed been an area of substantial interest recently [431]. Reduction of tropinone to form the alkaloids tropine and pseudotropine, with the 3α- and 3β-stereochemistry of the resulting alcohol occurs in different solanaceous plants to different extents. The two enzymes responsible for these stereoselective reduction processes have now been isolated from hairy root transformed cell cultures of *Datura stramonium* and many of their properties and substrate selectivity clarified [432,433]. This follows earlier work on the tropine reductases from *Hyoscyamus niger* [434] and *Atropa belladonna* [435].

In the case of scopolamine and hyoscyamine, the unit which then couples with the tropan-3α-ol is (*S*)-tropic acid, whose biosynthesis has been much debated over the years [436,437] since Leete originally discovered that the biosynthetic precursor was phenylalanine [438]. Many routes for this conversion have been suggested and, through experimentation eliminated, including the intermediacy of phenylpyruvate [439] or cinnamate [440]. The earlier demonstrations of intact incorporation [441,442] have now been affirmed by carbon-13 labelling using phenyl[1,3-$^{13}C_2$] lactic acid, which on metabolism into tropic acid in *Datura stramonium* afforded a product with contiguous labelled carbons which could be directly observed [443]. The esterase which attaches this unit to the tropanol has not been isolated as yet, although acyl transferases which produce ester quinolizidine alkaloids in *Lupinus* [444, 445] and *Cytisus* [446] species have been isolated.

In vivo NMR spectroscopy has also been applied to the study of biosynthetic processes [447], mostly for the examination of sequences in primary metabolism. Now this technique has been applied for the first time using *in vivo* ^{15}N NMR to the study of alkaloid biosynthesis in *Datura stramonium* and *Nicotiana tabacum* [448]. As well as several primary metabolites containing N, hyoscyamine was detected in *D. stramonium* and nicotine in *N. tabacum*, hydroxycinnamoyl putrescines were detected in both systems. Once further ^{15}N resonances for metabolites and intermediates, as well as the products, are assigned, this technique may offer great promise for time course studies on the ebb and flow of alkaloid biosynthesis.

NMR has also been applied to the determination of the level of acetylation of cell walls [449], an important factor in considering the digestibility of arabinoxylans in grasses [450] and of xyloglucans in legumes [451]. The procedure involves hydrolysis with 25% D_2SO_4–D_2O followed by ^1H NMR analysis of the total hydrolysate and integration of the acetic acid resonance and an ethanol standard resonance.

The dream of many natural product chemists, one is almost tempted say alchemists, is to take a simple precur-

sor, add a bunch of enzymes, wait, and isolate a desired natural product. Scott's remarkable work on the biosynthetic pathway of corrins makes this dream come true [452]. The pathway to vitamin B_{12} involves over 20 enzymes, and Scott has expressed many of the genes encoding the enzymes involved in the pathway. It was found that some of these could be combined in a single flask to produce precorrin-3x [453] and precorrin-5 [454]. In the latter case, the eight enzymes necessary for the nine-step conversion of aminolevulinic acid (ALA) to precorrin-5 were incubated with ALA and SAM to afford the desired corrin in 30% yield. As Scott suggested, the multienzyme synthesis of complex natural products has come of age [452].

A good demonstration of the application of physical chemistry techniques to the study of secondary metabolism comes from a study of one of the most fundamental reactions in all of nature, the oxidative polymerization of phenols, which is epitomized by the dimerization of coniferyl alcohol. Although this is thought to proceed through a radical coupling process, experimental evidence for this was lacking. Electrochemistry and pulse radiolysis have now been applied in aqueous and non-aqueous media to this reaction and the coniferyl radical has been observed and shown to dimerize by a radical–radical coupling [455]. The first step in this process is formation of phenolate anion which then undergoes one-electron oxidation to the phenoxyl radical.

Another important enzyme has also been isolated and characterized, namely the enzyme needed for the formation of the worlds' most consumed alkaloid, caffeine, i.e. *S*-adenosyl-L-methionine:theobromine 1-*N*-methyltransferase (STM) [456]. Although this enzyme was partially purified previously [457], the new procedure represents a higher degree of purification which allowed for an initial determination of the N-terminal sequence of the enzyme.

As occurred with the isolation and introduction into therapy of the *Catharanthus* alkaloids [458], research on taxol has burgeoned into many different areas of science. Some of these have already been touched upon (storage conditions, isolation of new analogs, synthesis, semisynthesis, etc.). Biosynthetic studies are also now being reported [47,459], especially given that very little is known about the biogenetic pathway of the taxane system [460]. In addition, as occurred in other areas of biosynthesis, e.g. indole alkaloids [461], isolation work on *Taxus* species may afford biogenetic clues to potential intermediates in the biosynthetic pathway. One of these, canadensene, which is functionalized at all of the appropriate carbons as taxol, except for C-1, and yet lacks the B, C and D rings of taxol, was recently characterized from the needles of *Taxus canadense* [462]. This would imply that at least in one species, functionalization of the partially cyclized skeleton can occur, rather than the multiple oxidation steps occurring on a preformed (A, B, and C rings intact) hydrocarbon [460].

Zenk has reviewed the importance of tissue culture preparations as a source of the enzymes of secondary metabolism [463], and has recognized that these enzymes have the potential to be integrated into the production of secondary metabolites either wholly, or as a part of a chemo-enzymatic synthesis of crucial natural products. That in itself may be crucial technology in the years ahead as certain resources become depleted. The key is the isolation of the gene which encodes the enzyme and the overexpression in an appropriate host system of its cDNA. Strictosidine synthase [464,465] and the berberine bridge enzyme [466,467] have now been expressed in the cell cultures of the fall army worm, *Spodoptera frugiperda*, using a baculovirus-based expression system [468]. Yields were approximately 4 mg of enzyme per l of cell culture, and the enzyme was secreted in active form into the medium, offering some advantages over the system expressed in *E. coli*. The work on strictosidine, from its original discovery by Smith, to its enzymatic formation and the expression of the gene for strictosidine synthase, make a fascinating story which has been elegantly reviewed by Kutchan [402].

SUMMARY

This brief review has attempted to look at how many of the strategies for the conduct of natural product research have changed in the recent past and how these changes have impacted the type of studies which are now being conducted by examining the work published in three journals in the field which have somewhat different foci. It is apparent through this review that the pace of natural products research and the level of global interest in this particular area of our environment has risen dramatically in the past few years. This trend is projected to continue for the future as the interface between chemistry and biology becomes even more blurred and the public demand rises for cost effective medications and biological agents from sustainable resources.

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REFERENCES

1. Farnsworth, N. R. and Morris, R. W. (1976) *Am. J. Pharmacol.* **147**, 46.
2. Farnsworth, N. R. (1977) in *Crop Resources* (Seigler, D. S., ed), p. 61. Academic Press, New York.
3. Farnsworth, N. R. (1979) *Am. J. Pharmacol. Educ.* **43**, 239.
4. Phillipson, J. D. (1985) *Pharmacol. J.* 334.
5. Tyler, V. E. (1986) *Econ. Bot.* **40**, 279.
6. Cordell, G. A. (1990) *Pharmacia* **30**, 169.
7. Cordell, G. A., Beecher, C. W. and Pezzuto, J. M. (1991) *J. Ethnopharmacol.* **32**, 117.

8. Cobb, R. R., Thornton, N. M., McGivney, R., Josephson, F., Wani, M. C., Wall, M. E. and Cordell, G. A. (1993) *Comments Toxicol.* **4**, 417.
9. Cordell, G. A. (1993) *Chem. Ind.* (London) 841.
10. Cordell, G. A. (1993) in *Studies in Natural Products Chemistry*, (Vol. 13: Bioactive Natural Products (Part A)) (Atta-ur-Rahman and Basha, F. Z., eds), p. 629. Elsevier Science Publishers, Amsterdam.
11. Cordell, G. A., Farnsworth, N. R., Beecher, C. W. W., Kinghorn, A. D., Pezzuto, J. M., Wall, M. E., Wani, M. C., Brown, D. M., Harris, T. J. R., Lewis, J. A., O'Neill, M. J. and Tait, R. M. (1993) in *Human and Medicinal Agents from Plants.* (Kinghorn, A.D. and Balandrin, M. F., eds), p. 191. American Chemical Society Symposium Series No. 534, Washington, DC.
12. Cordell, G. A., Farnsworth, N. R., Beecher, C. W. W., Kinghorn, A. D., Pezzuto, J. M., Wall, M. E., Wani, M. C., Cobb, R. R., Harris, T. J. R., O'Neill, M. J. and Tait, R. M. (1994) in *Anticancer Drug Discovery and Development: Natural Products and New Molecular Models.* (Valeriote, F. A., Corbett, T. H. and Baker, L. H., eds), p. 63. Kluwer Academic Publishers, Norwell, MA.
13. Cordell, G. A. (1995) in *Chemistry of the Amazon.* (Seidel, P. F., Gottlieb, O. R. and Colho Kaplan, M. A., eds), p. 8. American Chemical Society Symposium Series, No. 588, Washington, DC.
14. Kinghorn, A. D., Farnsworth, N. R., Beecher, C. W. W., Cordell, G. A., Pezzuto, J. M., Wall, M. E., Wani, M. C., Brown, D. M., O'Neill, M. J., Lewis, J. A., Tait, R. M., Besterman, J. M. and Harris, T. J. R. (1995) *Int. J. Pharmacog.* (Suppl.) (In press).
15. Cordell, G. A. (1995) *J. Ethnopharmacol.* (In press).
16. Cordell, G. A. (1995) *Proceedings of the Second Princess Chulabhorn Science Congress.* Chulabhorn Research Institute, Bangkok, Thailand (In press).
17. Hamburger, M. O. and Hostettmann, K. (1991) *Phytochemistry* **30**, 3864.
18. Farnsworth, N. R. (1980) *J. Ethnopharmacol.* **2**, 173.
19. Balandrin, M. F., Klocke, J. A., Wurtele, E. S. and Bollinger, W. H. (1991) *Science* **228**, 1154.
20. Verpoorte, R. (1989) *J. Ethnopharmacol.* **25**, 43.
21. Soejarto, D. D. and Farnsworth, N. R. (1989) *Perspect. Biol. Med.* **32**, 244.
22. Abelson, P. H. (1990) *Science* **247**, 513.
23. Wallace, J. (1991) *The World Book Health & Medical Annual*, p. 157. World Book, Inc., Chicago, IL.
24. Walton, N. J. (1992) *Chem. Brit.* 525.
25. Huxtable, R. J. (1992) *J. Ethnopharmacol.* **37**, 1.
26. Koskinen, A. M. P. (1993) *Asymmetric Synthesis of Natural Products.* John Wiley & Sons, Chichester.
27. Atta-ur-Rahman and Basha, F. Z. (eds) (1993) Studies in Natural Product Chemistry (Vol. 13: Bioactive Natural Products (Part A)) Elsevier Science Publishing, New York.
28. Mann, J. (1992) *Murder, Magic, and Medicine.* Oxford University Press, Oxford.
29. Herz, W., Kirby, G. W., Moore, R. E., Steglich, W. and Tamm, Ch. (eds) (1993) *Progress in the Chemistry of Natural Products* (Vol. 61). Springer-Verlag, Vienna.
30. Atta-ur-Rahman (ed.) (1992) *Advances in Natural Product Chemistry.* Harwood Academic Publishers, Chur, Switzerland.
31. Kinghorn, A. D. and Balandrin, M. F. (eds) (1993) *Human Medicinal Agents from Plants.* American Chemical Society Symposium Series No. 534, Washington, DC.
32. Harborne, J. B. and Baxter, H. (eds) (1993) *Phytochemical Dictionary.* Taylor & Francis, Bristol, PA.
33. Buckinghamn, J., Ayres, D. C., Bycroft, B. W., Collins, P. M., Gunstone, F. D., Harborne, J. B., Haslam, E., Hill, R. A., Kelly, D. R., Leeper, F. J., Murray, R. D. H. and Southon, I. W. (eds) (1994) *Dictionary of Natural Products.* Chapman and Hall, New York.
34. Pettit, G. R., Pierson, F. H. and Herald, C. L. (1994) *Anticancer Drugs from Animals, Plants and Microorganisms.* John Wiley & Sons, New York.
35. Haslam, E. (1993) *Shikimic Acid—Metabolism and Metabolites.* John Wiley & Sons, New York.
36. Atta-ur-Rahman (ed.) (1994) *Handbook of Natural Products Data* (Vol. 3: Isoquinoline Alkaloids.) Elsevier Science Publishing, Amsterdam.
37. Huang, K. -C. (1993) *The Pharmacology of Chinese Herbs.* CRC Press, Boca Raton, FL.
38. Ahmad, V. U. and Atta-ur-Rahman (eds) (1994) *Handbook of Natural Products Data* (Vol. 2: Pentacyclic Triterpenoids). Elsevier Science Publishing, Amsterdam.
39. Morrison, I. M. (ed) (1992) *Advances in Plant Cell Biochemistry and Biotechnology* (Vol. 1). Jai Press, London.
40. Lea, P. J. (ed) (1993) *Enzymes of Secondary Metabolism, Methods in Plant Biochemistry* (Vol. 9). Academic Press, London.
41. Bajaj, Y. P. S. (ed) (1993) *Biotechnology in Agriculture and Forestry.* Springer Verlag, Berlin.
42. Harborne, J. B. (ed) (1994) *The Flavonoids, Advances in Research Since* 1986. Chapman and Hall, London.
43. Cambie, R. C. and Ash, J. (1994) *Fijian Medicinal Plants.* CSIRO Publications, Victoria, Australia.
44. van Beek, T. A. and Breteler, H. (eds) (1993) *Phytochemistry and Agriculture.* Clarendon Press, Oxford.
45. Potemesil, M. and Pinedo, H. (eds) (1995) *Camptothecins: New Anticancer Agents.* CRC Press, Boca Raton, FL.
46. Suffness, M. (ed) (1995) *Taxol.* CRC Press, Boca Raton, FL.
47. Georg, G. I., Chen, T. T., Ojima, and Vyas, D. M. (eds) (1995) *Taxane Anticancer Agents.* American Chemical Society Symposium Series No. 583. Washington, DC.
48. Gore, A. (1993) *Earth in the Balance.* Penguin Books, New York.
49. Farnsworth, N. R., Akerele, O., Bingel, A. S.,

Soejarto, D. D. and Guo, Z. (1985) *Bull. WHO* **63**, 965.
50. Roach, M. (1993) *Discover* **November**, 58.
51. Gentry, A. (1993) in *Human Medicinal Agents from Plants*. (Balandrin, M. and Kinghorn, A. D., eds), p. 13. American Chemical Society Symposium Series 534, Washington, DC.
52. (1992) *Global Biodiversity Strategy*. WRI/IUCN/UNEP, Baltimore, MD.
53. Reid, W. V., Laird, S. A., Meyer, C. A., Gámez, R., Sittenfeld, A., Janzen, D. H., Gollin, M. A. and Juma, C. (eds) (1993) *Biodiversity Prospecting*. WRI, Baltimore, MD.
54. Wilson, E. O. (ed) (1988) *Biodiversity*. National Academy Press, Washington, DC.
55. Akerele, O., Heywood, V. and Synge, H. (eds) (1991) *Conservation of Medicinal Plants*. Cambridge University Press, Cambridge.
56. Farnsworth, N. R. (1990) in *Bioactive Compounds from Plants*, p. 2. Ciba Foundation Symposium 154, Wiley, Chichester.
57. Balick, M. J. (1990) in *Bioactive Compounds from Plants*, p. 22. Ciba Foundation Symposium 154, Wiley, Chichester.
58. Cox, P. A. (1990) in *Bioactive Compounds from Plants*, p. 40. Ciba Foundation Symposium 154, Wiley, Chichester.
59. Soejarto, D. D. (1993) in *Human Medicinal Agents from Plants*. (Balandrin, M. and Kinghorn, A. D., eds), p. 96. American Chemical Society Symposium Series No. 534, Washington, DC.
60. (1994) *Ethnobotany and the Search for New Drugs*. Ciba Foundation Symposium 185, Wiley, Chichester.
61. King, S. R. and Tempesta, M. S. (1994) in *Ethnobotany and the Search for New Drugs* p. 197. Ciba Foundation Symposium 185, Wiley, Chichester.
62. King, S. R. (1994) in *Intellectual Property Rights for Indigenous Peoples, A Sourcebook* (Greaves, T., ed), p. 69. Society for Applied Anthropology, Oklahoma City, OK.
63. Phillipson, J. D. and Anderson, L. A. (1989) *J. Ethnopharmacol.* **25**, 61.
64. Cox, P. A. and Balick, M. J. (1994) *Sci. Amer.* **June**, 42.
65. Holland, B. K. (1994) *Nature* **369**, 702.
66. Bruhns, A. (1994) *The Ottawa Citizen* **April 16**, p. A6.
67. Lietava, J. (1992) *J. Ethnopharmacol.* **35**, 263.
68. DeWitt, S. H. (1994) *Pharm. News* **1**, 11.
69. Houghton, R. A. (1994) *Curr. Biol.* **4**, 564.
70. Nielsen, J. (1994) *Chem. Ind.* (London) 902.
71. Schiff, P. B., Fant, J. and Horwitz, S. B. (1979) *Nature* (London) **277**, 665.
72. Schiff, P. B. and Horwitz, S. B. (1980) *Proc. Natl. Acad. Sci. USA*, **77**, 1561.
73. Parness, J. and Horwitz, S. B. (1981) *J. Cell Biol.* **91**, 479.
74. Manfredi, J. J., Parness, and Horwitz, S. B. (1982) *J. Cell Biol.* **94**, 688.
75. Kingston, D. G. I., Samaranayake, G. and Ivey, C. A. (1990) *J. Nat. Prod.* **53**, 1.
76. Rowinsky, E. K., Cazenave, L. A., and Donehower, R. C. (1990) *J. Natl. Cancer Inst.* **82**, 1247.
77. Kingston, D. G. I. (1991) *Pharmacol. Ther.* **52**, 1.
78. Rowinsky, E. K., Onetto, N., Canetta, R. M., and Arbuck, S. G. (1992) *Sem. Oncol.* **19**, 646.
79. Horwitz, S. B. (1992) *Trends Pharmacol. Sci.* **13**, 134.
80. Kingston, D. G. I. (1993) in *Human Medicinal Agents from Plants*. (Kinghorn, A. D. and Balandrin, M.F., eds), p. 138. American Chemical Society Symposium Series No. 534, Washington, DC.
81. Guénard, D., Guéritte-Voegelein, F. and Potier P. (1993) *Acc. Chem. Res.* **26**, 160.
82. Kingston, D. G. I., Molinero, A. A. and Rimoldi, J. M. (1993) *Prog. Chem. Org. Nat. Prod.* **61**, 1.
83. Joel, S. P. (1994) *Chem. Ind.* (London) 172.
84. Nicolaou, K. C., Dai, W. -M., and Guy, R. K. (1994) *Angew. Chem.* **106**, 38.
85. Kingston, D. G. I. (1994) *Trends Biotechnol.* **12**, 222.
86. Wani, M. C., Taylor, H. L., Wall, M. E., Coggon, P. and McPhail, A. T. (1971) *J. Am. Chem. Soc.* **93**, 2325.
87. McGuire, W. P., Rowinsky, E. K., Rosenshein, N. B., Grumbine, F. C., Ettinger, D. S., Armstrong, D. K. and Donehower, R. C. (1989) *Ann. Int. Med.* **111**, 273.
88. Chase, M. (1991) *Wall Street J.* **April 19**, A1, A8.
89. Anon. (1992) *Audubon* **March**, 80.
90. Borman, S. (1991) *Chem. Eng. News* **September 2**, 11.
91. Nicolaou, K. C., Yang, Z., Liu, J. -J., Ueno, Nantermet, P. G., Guy, R. K., Claiborne, C. F., Renaud, J., Couladouros, E. A., Paulvannan, K. and Sorenson, E. J. (1994) *Nature* (London) **367**, 630.
92. Nicolaou, K. C., Ueno, H., Liu, J. -J., Nantermet, P. G., Yang, Z., Renaud, J., Paulvannan, K. and Chadha, R. (1995) *J. Am. Chem. Soc.*, **117**, 653.
93. Nicolaou, K. C. and Guy, R. K. (1995) in *Taxane Anticancer Agents* (Georg, G. I., Chen, T. T., Ojima, and Vyas, D. M., eds), p. 302. American Chemical Society Symposium Series No. 538, Washington, DC.
94. Holton, R. A., Kim, H. -B., Somoza, C., Liang, F., Biediger, R. J., Boatman, P. D., Shindo, M., Smith, C. C., Kim, S., Nadizeh, H., Suzuki, Y., Tao, C., Vu, P., Tang, S., Zhang, P., Murthi, K. K., Gentile, L. N. and Liu, J. H. (1994) *J. Am. Chem. Soc.* **116**, 1599.
95. Holton, R. A., Somoza, C., Kim, H. -B., Liang, F., Biediger, R. J., Boatman, P. D., Shindo, M., Smith, C.C., Kim, S.-C. (1995) in *Taxane Anticancer Agents*. (Georg, G. I., Chen, T. T., Ojima, and Vyas, D. M., eds), p. 288. American Chemical Society Symposium Series No. 538, Washington, DC.
96. Wender, P. A., Badman, N. F., Conway, S. P., Floreancig, P. E., Glass, T. E., Houze, J. B., Krauss, N. E., Lee, D. -S. and Marquess, D. G. (1995) in *Taxane Anticancer Agents*. (Georg, G. I., Chen, T. T., Ojima, and Vyas, D. M., eds), p. 326. American

Chemical Society Symposium Series No. 538, Washington, DC.
97. Denis, J. -N., Greene, A., Guénard, D., Guérritte-Voegelin, F, Mangatal, L. and Potier, P. (1988) *J. Am. Chem. Soc.* **110**, 5917.
98. Vance, N. C., Kelsey, R. G. and Sabin, T. E. (1994) *Phytochemistry* **36**, 1241.
99. Schutzki, R. E., Chandra, A. and Nair, M. G. (1994) *Phytochemistry* **37**, 405.
100. ElSohly, H. N., Croom, E. C., El-Kashoury, E., ElSohly, M. A. and McChesney, J. D. (1994) *J. Nat. Prod.* **57**, 1025.
101. McClure, T. D., Schram, K. H. and Reimer, M. L. J. (1992) *J. Am. Mass Spectrom.* **3**, 672.
102. Gimon, M. E., Kinsel, G. R. and Russell, D. H. (1993) in *Proceedings of the 41st ASMS Conference on Mass Spectrometry and Allied Topics*, p. 412a. San Francisco, CA (cited in Ref. 107).
103. Auriola, S. O. K., Lepistö, A. -M., Naaranlathi, T. and Lapinjoki, S. P. (1992) *J. Chromatogr.* **594**, 153.
104. Bitsch, J., Ma, W., MacDonald, F., Neider, M. and Shakleton, C. H. L. (1993) *J. Chromatogr.* **615**, 273.
105. Mattina, M. J. I ., Giordano, G. and McMurray, W.J. (1992) in *Proceedings of the 40th ASMS Conference on Mass Spectrometry and Allied Topics*, p. 894. Washington, DC (cited in Ref. 107).
106. Jaziri, M., Diallo, B. M., Vanhaelen, M. H., Vanhaelen-Fastre, R. J., Zhiri, A., Becu, A. G. and Homes, J. (1991) *J. Pharmacol Belg.* **46**, 93.
107. Hike II, S. H., Cooks, R. G., Chang, C. -J., Kelly, R. C., Qualls, S. J., Alvarado, B., McGuire, M. T. and Snader, K. M. (1994) *J. Nat. Prod.* **57**, 277.
108. Kerns, E. H., Volk, K. J., Hill, S. E. and Lee, M. S. (1994) *J. Nat. Prod.* **37**, 1391.
109. Boyd, M. R. (1989) *Proc. Am. Assoc. Cancer Res.* **30**, 652.
110. Boyd, M. R. (1988) in *AIDS Etiology, Diagnosis, Treatment and Prevention* (DeVita, V. T. Jr, Hellman, S. and Rosenberg, S. A., eds), p. 305, Lippincott, Philadelphia, PA..
111. Cragg, G. M., Boyd, M. R., Cardellina, II, J. H., Grever, M. R., Schepartz, S. A., Snader, K. M. and Suffness, M. (1993) in *Human Medicinal Agents from Plants.* (Kinghorn, A.D. and Balandrin M.F., eds), p. 80. American Chemical Society Symposium Series No. 534, Washington, DC.
112. Cardellina, II, J. H., Gustafson, K. R., Beutler, J. A., McKee, T. C., Hallock, Y. F., Fuller, R. W. and Boyd, M. R. (1993) in *Human Medicinal Agents from Plants.* (Kinghorn, A.D. and Balandrin, M.F., eds), p. 218. American Chemical Society Symposium Series No. 534, Washington, DC.
113. Sneader, W. (1985) *Drug Discovery: The Evolution of Modern Medicines*, p. 296. John Wiley & Sons, Chichester.
114. Hileman, B. (1992) *Chem. Eng. News* July 6, 7.
115. Anon (1992) *WRI/IUCN/UNEP* **3**(2) 1.
116. Baker, J. T., Borris, R. P., Carte, B., Cordell, G. A., Soejarto, D. D., Cragg, G. M., Gupta, M. P., Iwu M. M. and Tyler, V. E. (1995) *J. Nat. Prod.* (In press).
117. Lovelock, J. E. (1987) *Gaia. A New Look at Life on Earth (2nd Edn)* . Oxford University Press, Oxford.
118. Kashman, Y., Gustafson, K. R., Fuller, R. W., Cardellina, II, J. H., McMahon, J. B., Currens, M. J., Buckheit Jr, R. W., Hughes, S. H., Cragg, G.M. and Boyd, M. R. (1992) **35**, 2735.
119. Dawes, K. O. (1992) *Chicago Sun-Times* **November 9**, 20.
120. Fuller, R. W., Bokesch, H. R., Gustafson, K. R., McKee, Cardellina, II, J. H., McMahon, J. B., Cragg, G. M., Soejarto, D. D. and Boyd, M. R. (1994) *Bioorg. Med. Chem. Lett* **4**, 1961.
121. Patil, A. D., Freyer, A. J., Eggleston, D. S., Haltiwanger, R. C., Bean, M. F., Taylor, P. B., Caranfa, M. J., Breen, A. L., Bartus, H. R., Johnson, R. K., Hertzberg, R. P. and Westley, J. W. (1993) *J. Med. Chem.* **36**, 4131.
122. Shenon, P. (1994) *NY Times* **December 6**, B9.
123. Bringmann, G. and Pokorny, F. (1995) *The Alkaloids*, (Vol. 46) (Cordell, G.A., ed), p. 128. Academic Press, San Diego, CA.
124. Manfredi, K. P., Blunt, J. W., Cardellina II, J. H., McMahon, J. B., Pannell, L. L., Cragg, G. M. and Boyd, M. R. (1991) *J. Med. Chem.* **34**, 3402.
125. Boyd, M. R., Halloch, Y. F., Cardellina, III, J. H., Manfredi, K. P., Buckheit, Jr., R. W., Bringmann, G. and Schaeffer, M. (1994) *J. Med. Chem.* **37**, 1740.
126. Thomas, D. W. and Gereau, R. E. (1993) *Novon* **3**, 494.
127. Bringmann, G., Zagst, R., Schäffer, M., HallocK, Y. F., Manfredi, K. P. and Cardellina, II, J. H. (1993) *Angew. Chem.* **105**, 1242.
128. Bringmann, G., Harmsen, S., Holenz, J., Geuder, T., Götz, R., Keller, P. A., Walter, R., Hallock, Y. F., Cardellina, II, J. H. and Boyd, M. R. (1994) *Tetrahedron* **50**, 9643.
129. Kelly, T. R., Garcia, A., Lang, F., Walsh, J. J., Bhaskar, K. V., Boyd, M. R., Götz, R., Keller, P. A., Walter, R. and Bringmann, G. (1994) *Tetrahedron Lett.* **35**, 7621.
130. Hoye, T. R., Chen, M. -Z., Mi, L. and Priest, O. P. (1994) *Tetrahedron Lett.* **35**, 8747.
131. McMahon, J. B., Currens, M. J., Gulakowski, R. J., Buckheit, Jr., R. W., Lackman-Smith, C., Hallock, Y. F. and Boyd, M. R. (1995) *Antimicrob. Agents Chemother.* **39**, 484.
132. Beecher, C. W. W and Constant, H. (1995) *Nat. Prod. Letts.* **6**, 193.
133. Corley, D. G. and Durley, R. C. (1994) *J. Nat. Prod.* **57**, 1484.
134. Beutler, J. A., McKee, T. C., Fuller, R. W., Tischler, M., Cardellina II, J. H., McCloud, T. G., Snader, K. M. and Boyd, M. R. (1993) *Antiviral Chem. Chemother.* **4**, 167.
135. Cardellina, II, J. H., Munro, M. H. G., Fuller, R. W., Manfredi, K. P., McKee, T. C., Tischler, M., Bokesch, H. R., Gustafson, K. R., Beutler, J. A. and Boyd, M. R. (1993) *J. Nat. Prod.* **56**, 1123.
136. Baba, M., Snoek, R., Paulweis, R. and DeClerq, E. (1988) *Antimicrob. Agents. Chemother.* **32**, 1742.

137. Haslam, E. (1979) in *Biochemistry of Plant Phenolics* (Swain, T., Harborne, J. B. and Van Sumere, C. F. eds), p. 475. Plenum Press, New York.
138. Wilson, J. A., and Merrill, H. B. (1931) in *Analysis of Leather and Materials Used in Making It*, p. 290. McGraw-Hill, New York.
139. Loomis, W. D. and Battaile, J. (1966) *Phytochemistry* **5**, 423.
140. Wall, M. E., Taylor, H., Amrosio, L., and Davis, K. (1969) *J. Pharm. Sci.* **58**, 839.
141. Tan, G. T., Pezzuto, J. M., Kinghorn, A. D. and S. H. Hughes. (1991) *J. Nat. Prod.* **54**, 143.
142. Brabley, S., Hammann, P., Kluge, H., Wink, J., Kricke, P. and Zeeck, A. (1991) *J. Antibiot.* **44**, 797.
143. Noltemeyer, M., Sheldrick, G. M., Hoppe, M. -V. and Zeeck, A. (1982) *J. Antibiot.* **35**, 549.
144. Ikan, R. (1991) *Natural Products. A Laboratory Guide*. Academic Press, San Diego, CA.
145. Paull, K., Shoemaker, R. H., Hodes, L., Monks, A., Scudiero, D. A., Rubinstein, L., Plowman, J. and Boyd, M. R. (1989) *J. Natl. Cancer Inst.* **81**, 1088.
146. Fuller, R. W., Cardellina II, J. H., Cragg, G. M. and Boyd, M. R. (1994) *J. Nat. Prod.* **57**, 1442.
147. Marston, A., Slacanin, I. and Hostettmann, K. (1990) *Phytochem. Anal.* **1**, 3.
148. Rahalison, L., Hamburger, M., Hostettmann, K., Monod, M. and Frenk, E. (1991) *Phytochem. Anal.* **2**, 199.
149. Hamburger, L. and Cordell, G. A. (1987) *J. Nat. Prod.* **50**, 19.
150. Kato, A., Moriyasu, M., Ichimaru, M. M., Nishiyama, Y., Juma, F. D., Nganga, J. N., Mathenge, S. G. and Ogeto, J. O. (1995) *Phytochem. Anal.* **6**, 89.
151. *Drug Stereochemistry: Analytical Methods and Pharmocology*. Wainer, I. W. and Drayer, D. E. (eds) (1993) Marcel Dekker, New York.
152. Stinson, S. C. (1992) *Chem. Engng. News* **Sept. 28** 46.
153. Brossi, A. (1993) in *The Alkaloids*, (Vol. 43) (Cordell, G.A., eds), p. 119. Academic Press, San Diego, CA.
154. Waller, D. P., Bunyapraphatsara, N., Martin, A., Vournazos, R. J., Ahmed, M. M., Soejarto, D. D., Cordell, G. A., Fong, H. H. S., Russell, L. D. and Malone, J. P. (1983) *J. Andrology* **4**, 276.
155. Kim, I. C., Waller, D. P., Marcelle, G. B., Cordell, G. A., Fong, H. H. S., Pirkle, W. H., Pilla, L. and Matlin, S. A. (1984) *Contraception* **30**, 253.
156. Pirkle, W. H., Fin, J. M., Schiener, J. L. and Hamper, B. C. *J. Am. Chem. Soc.* **103**, 3964.
157. Köpke, T., Schmarr, H. -G. and Mosandl, A. (1992) *Flav. Frag. J.* **7**, 205.
158. Karl, V., Rettinger, K., Dietrich, H. and Mosandl, A. (1993) *Dentsch. Lebensm. Rundsch.* **88**, 147.
159. Köpke, T., Dietrich, A. and Mosandl, A. (1994) *Phytochem. Anal.* **5**, 61.
160. Karl, V. Dietrich, A. and Mosandl, A. (1994) *Phytochem. Anal.* **5**, 32.
161. Nikolova–Damyanaova, B., Ilieva, E., Handjieva, N. and Bankova, V. (1994) *Phytochem. Anal.* **5**, 38.
162. Gränicher, F., Christen, P. and Vuagnat, P. (1994) *Phytochem. Anal.* **5**, 297.
163. Zhang, L., Ying, H., Zhang, Z. -X., Sheng, L. -S. and An, D. -K. (1994) *Phytochem. Anal.* **5**, 141.
164. Brown, M. S., Molyneux, R. J. and Roitman, J. N. (1994) *Phytochem. Anal.* **5**, 251.
165. Giddings, J. C. (1989) *J. Chromatogr.* **480**, 21.
166. Landers, J. P., Oda, R. P., Spelsberg, T. C., Nolan, J. A. and Ulfelder, K. J. (1993) *BioTechniques* **14**, 98.
167. McGhie, T. K. and Markham, K. R. (1994) *Phytochem. Anal.* **5**, 121.
168. Pietta, P. G., Mauri, P. L., Gardana, C. and Zini, L. (1994) *Phytochem. Anal.* **5**, 305.
169. Liu, Y. -M., Sheu, S. -J., Chiou, S. -H., Chang, H. -C. and Chen, Y. -P. (1994) *Phytochem. Anal.* **5**, 256.
170. Wolfender, J. -L., Maillard, M. and Hostettmenn, K. (1994) *Phytochem. Anal.* **5**, 153.
171. Verpoorte, R. and Niessen, W. M. A. (1994) *Phytochem. Anal.* **5**, 217.
172. Scheller, G., Stahl, B., Weyd, S., Hillenkamp, F. and Schreier, P. (1994) *Phytochem. Anal.* **5**, 281.
173. Cordell, G. A., and G. Blaskó. (1989) in *Studies in Natural Products Chemistry* (Vol. 5: Structure Elucidation (Part B)). (Atta-ur-Rahman, ed), p. 1. Elsevier, Amsterdam.
174. Cordell, G. A. (1991) *Phytochem. Anal.* **2**, 59.
175. Cordell, G. A. and Kinghorn, A. D. (1991) *Tetrahedron.* **47**, 3521.
176. Bax, A. (1982) *Two-Dimensional Nuclear Magnetic Resonance in Liquids*. Delft University Press, Delft, The Netherlands.
177. Derome, A. E. (1987) *Modern NMR Techniques for Chemistry Research*. Pergamon Press, Oxford.
178. Martin, G. E. and Zektzer, A. S. (1988) *Two-Dimensional NMR Methods for Establishing Molecular Connectivity*. VCH Publishers, Inc., New York.
179. Atta-ur-Rahman (1986) *Nuclear Magnetic Resonance: Basic Principles*. Springer, Berlin.
180. Derome, A. E. (1989) *Nat. Prod. Rep.* **6**, 111.
181. Kriwacki, R. W. and Pitner, T. P. (1989) *Pharmaceut. Res.* **6**, 531.
182. Aue, W. P., Bartholdi, E., and Ernst, R. R. (1976) *J. Chem. Phys.* **64**, 2229.
183. Bax, A. and Freeman, R. (1981) *J. Magn. Reson.* **44**, 542.
184. Piantini, U., Sørenson, O. W. and Ernst, R. R. (1982) *J. Am. Chem. Soc.* **104**, 6800.
185. Rance, M., Sørenson, O. W., Bodenhausen, G., Wagner, G., Ernst, R. R. and Wuthrich, K. (1983) *Biochem. Biophys. Res. Commun.* **117**, 479.
186. Braunschweiler, L. and Ernst, R. R. (1983) *J. Magn. Reson.* **53**, 521.
187. Bax, A. and Davis, D. G. (1985) *J. Magn. Reson.* **65**, 355.
188. Davis, D. G. and Bax, A. (1985) *J. Am. Chem. Soc.* **107**, 2820, 7197.
189. Edwards, M. W. and Bax, A. (1986) *J. Am. Chem. Soc.* **108**, 918.
190. Jeener, J., Meier, B. H., Bachman, P. and Ernst, R. R. (1979) *J. Chem. Phys.* **71**, 4546.

191. Bothner-By, A. A., Stephens, R. L., Lee, J., Warren, C. D. and Jeanloz, R. W. (1984) *J. Am. Chem. Soc.* **106**, 811.
192. Summers, M. F., Marzilli, L. G. and Bax, A. (1986) *J. Am. Chem. Soc.* **108**, 4285.
193. Griesinger, C. and Ernst, R. R. (1987) *J. Magn. Reson.* **75**, 261.
194. Bodenhausen, G. and Freeman, R. (1978) *J. Am. Chem. Soc.* **100**, 320.
195. Bax, A. and Morris, G.A. (1981) *J. Magn. Reson.* **42**, 501.
196. Bax., A. (1983) *J. Magn. Reson.* **53**, 517.
197. Bax, A. and Summers, M. F. (1986) *J. Am. Chem. Soc.* **108**, 2093.
198. Bax, A. and Subramanian, S. (1986) *J. Magn. Reson.* **67**, 565.
199. Bax, A. and Summers, M. F. (1986) *J. Am. Chem. Soc.* **108**, 2093.
200. Bax, A., Aszalos, A., Dinya, Z. and Sudo, K. (1986) *J. Am. Chem. Soc.* **108**, 8056.
201. Reynolds, W. F., McLean, S., Perpick-Dumont, M. and Enriguez, R. G. (1989) *Magn. Reson. Chem.* **27**, 162.
202. Carpenter, K. A., Reynolds, W. F., Yang, J.-P. and Enriquez, R. G. (1992) *Magn. Reson. Chem.* **30**, S35.
203. Bax, A. (1984) *J. Magn. Reson.* **57**, 314.
204. Kessler, H., Griesinger, C., Zarbock, J. and Loosli, H. R. (1984) *J. Magn. Reson.* **57**, 331.
205. Levitt, M. H. and Ernst, R. R. (1981) *Molec. Phys.* **50**, 1109.
206. Bax, A., Freeman, R. and Frenkeil, T. A. (1981) *J. Am. Chem. Soc.* **103**, 2102.
207. Byrne, L. T. (1993) in *Bioactive Natural Products. Detection, Isolation and Structure Determination* (Colegate, S.M. and Molyneux, R. J., eds), p. 75. CRC Press, Boca Raton, FL.
208. Lin, L. -Z., Lin, L. -J., Cordell, G. A., Luo, S. Q. and Jiang, H. -F. *Magn. Reson. Chem.* **30**, 1097.
209. Likhitwitayawuid, K., Bavovada, R., Lin, L. -Z. and G. A. Cordell, G. A. (1993) *Phytochemistry* **34**, 759.
210. Luo, S. -Q., Lin, L. -Z., Cordell, G. A., Xue, L. and Johnson, M. E. (1993) *Phytochemistry* **34**, 1615.
211. Gil, R. R., Lin, L. -Z., Chai, H. -B. Pezzuto, J. M. and Cordell, G. A. (1995) *J. Nat. Prod.* (In press).
212. Stuppner, H. and Müller, E. P. (1994) *Phytochemistry* **37**, 1483.
213. Gil, R. R., Cordell, G. A., Topçu, G. and Ulubelen, A. (1994) *J. Nat. Prod.* **57**, 181.
214. Murata, M., Naoki, N., Iwashita, T., Matsunaga, S., Sasaki, M., Yokoyama, A. and Yasumoto, T. (1993) *J. Am. Chem. Soc.* **115**, 2060.
215. Rupprecht, J. K., Hui, Y. -H. and McLaughlin, J. L. *J. Nat. Prod.* **43**, 947.
216. Fang, X. -P., Reiser, M., Gu, Z. -M., Zhao, G. -X. and McLaughlin, J. L. (1993) *Phytochem. Anal.* **4**, 27, 69.
217. Dale, J. A. and Mosher, H. S. (1973) *J. Am. Chem. Soc.* **95**, 512.
218. Ohtani, I., Kusumi, T., Kashman, Y. and Kakisawa, H. (1991) *J. Am. Chem. Soc.* **113**, 4092.
219. Rieser, M. T., Hui, Y. -H., Rupprecht, J. K., Kozlowski, J. F., Wood, K. V., Mclaughlin, J. L., Hanson. P. R., Zhuang, Z. and Hoye, T. R. (1992) *J. Am. Chem. Soc.* **114**, 10203.
220. Asada, Y., Ikeno, M. and Furuya, T. (1994) *Phytochemistry* **35**, 757.
221. Imamura, T., Watanabe, T., Kuwahara and Koshijima, T. (1994) *Phytochemistry* **37**, 1165.
222. Piveteau, D., Delsuc, M. -A. and Lallemand, J. -Y. (1985) *J. Magn. Reson.* **63**, 255.
223. O'Mathúna, D. P. Doskotch, R. W. (1994) *J. Nat. Prod.* **57**, 767.
224. Bax, A. and Davis, D. G. (1985) *J. Magn. Reson.* **65**, 355.
225. Kessler, H., Oschkinat, H. and Griesinger, C. (1986) *J. Magn. Reson.* **70**, 106.
226. Wessel, H. P., Englert, G. and Stangier, P. (1991) *Helv. Chim. Acta* **74**, 682.
227. Willker, W. and Liebfritz, D. (1992) *Magn. Reson. Chem.* **30**, 645.
228. Puri, R., Wong, T. -C. and Puri, R. K. (1994) *J. Nat. Prod.* **57**, 587.
229. Blumenthal, M. (1994) *Herbalgram* **32**, 18.
230. Cai, Y., Evans, F. J., Roberts, M. F., Phillipson, J. D., Zenk, M. H. and Gleba, Y.Y. (1991) *Phytochemistry* **30**, 2033.
231. Cai, Y., Phillipson, J. D., Harper, J. I. and Corne, S. J. (1994) *Phytochem. Anal.* **5**, 183.
232. Saito, N. Timberlake, C. F., Tucknott, O. G., and Lewis, I. A. S. (1983) *Phytochemistry* **22**, 1007.
233. Baj, A., Bombardelli, E., Gabetta, B., Martinelli, E. M. and Mustich, G. (1977) *J. Chromatogr.* **139**, 111.
234. Roepstorff, P. (1989) *Acc. Chem. Res.* **22**, 421.
235. Wood, K. V., Bonham, C., Hipskind, J. and Nicholson, R. L. (1994) *Phytochemistry* **37**, 557.
236. Massiot, G., Dijoux, M. -G., Lavaud, C., Le Men-Olivier, L., Connolly, J. D. and Sheeley, D. M. (1994) *Phytochemistry* **37**, 1667.
237. Mino, Y., Usami, H., Inoue, S., Ikeda, K. and Ota, N. (1993) *Phytochemistry* **33**, 601.
238. Mino, Y. (1994) *Phytochemistry* **35**, 385.
239. Mino, Y. (1994) *Phytochemistry* **37**, 429.
240. Hamburger, M. and Hostettmann, K (1991) *Phytochemistry* **30**, 3864.
241. O'Neill, M. J. and Lewis, J. A. (1993) in *Human Medicinal Agents from Plants* (Kinghorn, A. D. and Balandrin, M. F., eds), p. 48. American Chemical Society Symposium Series No. 534, Washington, DC.
242. Hostettmann, K. (ed) (1991) *Methods in Plant Biochemistry* (Vol. 6: Assays for Bioactivity) (a) p. 33, (b) p. 47, (c) p. 71, (d) p. 135, (e) p. 153, (f) p. 179, (g) p. 195, (h) p. 219, and (i) p. 235. Academic Press, London.
243. van Rossum, J. M. and Hurkmans, J. Th. A. (1986) *Pharmacological Methods – Receptors and Chemotherapy* (Parnham, M. J. and Bruinvels, J., eds), p. 63. Elsevier Science Publishers, Amsterdam.
244. Sweetnam, P. M., Caldwell, L., Lancaster, J., Bauer,

C. Jr., McMillan, B., Kinnier, W. J. and Price, C. H. (1993) *J. Nat. Prod.* **56**, 441.
245. Havsteen, B. (1993) *Biochem Pharmacol.* **32**, 1141.
246. Wall, M. E., Wani, M. C., Cooke, C. E., Palmer, K. H., McPhail, A. T. and Sim. G. A. (1966) *J. Am. Chem. Soc.* **88**, 3888.
247. Wall, M. E. and Wani, M. C., (1995) *Cancer Res.* **55**, 753.
248. Schaeppi, U., Fleischman, R. W. and Cooney, D. A. (1974) *Cancer Chemother. Rep. Part* 3 **5**, 25.
249. Hsieh, T., Yang, C. -C., Chang, H. -Y., Ling, K. -M. and Chao, P. -H. (1979) *Chin Med. J.* **92**, 57.
250. Hsiang, Y. -H., Hertzberg, R., Hecht, S. and Liu, L. (1985) *J. Biol. Chem.* **260**, 14873.
251. Hsiang, Y. -H. and Liu, L. F. (1988) *Cancer Res.* **48**, 1722.
252. Slichmeyer, W. J., Rowinsky, E. K., Donehower, R. C. and Kaufmann, S. H. (1993) *J. Nat. Cancer Inst.* **85**, 271.
253. Wall, M. E. and Wani, M. C. (1993) *Human Medicinal Agents from Plants* (Kinghorn, A. D. and Balandrin, M. F., eds), p. 149. ACS Symposium Series 534, Washington, DC.
254. Vyas, D. M. (1993) *Pharmacochem. Libr.* **20**, 261.
255. Kingston, D. G. I. (1994) *Trends Biotechnol.* **12**, 222.
256. Nicolaou, K. C., Dai, W. -M. and Guy, R. K. (1994) *Angew. Chem.* **106**, 38.
257. Pisha, E., Chai, H. -B., Lee, I. -S., Wickramaratne, M., Chagwedera, T. E., Twatchai, R., Soejarto, D. D., Farnsworth, N. R., Cordell, G. A., Beecher, C. W. W., Fong, H. H. S., Kinghorn, A. D., Brown, D. M., Wani, M. C., Wall, M. E., Heiken, T. J., Das Gupta, T. K. and Pezzuto, J. M. *Nature (Medicine)*, (In press).
258. Suffness, M. (1989) *Gann* **36**, 21 (and Refs therein).
259. Valeriote, F. A., Corbett, T. H. and Baker, L. H. (eds) (1994) *Anticancer Drug Discovery and Development: Natural Products and New Molecular Models.* Kluwer Academic Publishers, Norwell, MA.
260. Suffness, M., Cragg, G. M., Grever, M. R., Grifo, F. T., Johnson, G., Mead, J. A. R., Schepartz, S. A., Venditti, J. M. and Wolpert, M. (1995) *Int. J. Pharmacog.* (Suppl.) (in press).
261. Hamburger, M. O. and Cordell, G. A. (1987) *J. Nat. Prod.* **50**, 19.
262. Somanbandhu, A., Nitayangkura, S., Mahidol, C., Ruchirawat, S., Likhitwitayawuid, K., Cordell, G. A., Shieh, H. -L., Chai, H. -B. and Pezzuto, J. M. (1993) *J. Nat. Prod.* **56**, 233.
263. Saifah, E., Likhitwitayawuid, K. Puripattanavong, J., Cordell, G. A. Chai, H. -B. and Pezzuto, J. M. (1993) *J. Nat. Prod.* **56**, 473.
264. You, M., Ma, X., Mukherjee, R., Farnsworth, N. R., Cordell, G. A., Kinghorn, A. D. and Pezzuto, J. M. (1994) *J. Nat. Prod.* **57**, 1517.
265. You, M., Cordell, G. A. and Pezzuto, J. M. (1995) *J. Nat. Cancer Inst.* (Submitted).
266. You M., Wickramaratne, D. B. M., Silva, G. L., Chai, H. -B., Chagwedera, T. E., Farnsworth, N. R., Cordell, G. A., Kinghorn, A. D. and J. M. Pezzuto, J. M. (1995) *J. Nat. Prod.* **58**, 598.
267. Johnson, S. W., Ozols, R. F. and Hamilton, T. C. (1993) *Cancer* **71** (2 Suppl), 644.
268. McLaughlin, J. L. (1991) *Methods in Plant Biochemistry* (Vol. 6: Assays for Bioactivity) (Hostettmann, K., ed), p. 1. Academic Press, London.
269. McLaughlin, J. L., Chang, C. -J. and Smith D. L. (1991) in *Sidies in Natural Products Chemistry* (Vol. 9) (Atta-ur-Rahman, ed), p. 383. Elsevier, Amsterdam.
270. Meyer, B. N., Ferrigni, N. R., Putnam, J. E., Jacobson, L. B., Nichols, D. E. and McLaughlin, J. L. (1982) *Planta Med.* **45**, 31.
271. Ferrigni, N. R., Putnam, J. E., Anderson, B., Jacobsen, L. B., Nichols, D. E., Moore, D. S. and McLaughlin, J. L. (1982) *J. Nat. Prod.* **45**, 679.
272. Anderson, J. E., Goetz, C. M., McLaughlin, J. L. and Suffness, M. (1991) *Phytochem. Anal.* **2**, 102.
273. McLaughlin, J. L., Chang, C. -J, and Smith, D. L. (1993) *Human Medicinal Agents from Plants.* (Kinghorn, A. D. and Balandrin, M. F., eds), p. 112. American Chemical Society Symposium Series No. 534, Washington, DC.
274. Bryant, F. O., Cutler, H. G., Parker, S. R. and Jacyno, J. M. (1994) *J. Nat. Prod.* **57**, 640.
275. Ngassapa, O., Soejarto, D. D., Pezzuto, J. M. and Farnsworth, N. R. (1994) *J. Nat. Prod.* **57**, 1.
276. Ma. W. -W., Park, G. L., Gomez, G. A., Nieder, M. H., Adams, T. L., Aynsley, J. S., Sahai, O. P., Smith, R. J., Stahlbut, R. W., Hylands, P. J., Bitsch, F. and Shackleton, C. (1994) *J. Nat. Prod.* **57**, 116.
277. Kuo, Y. -H., King, M. -L., Chen, C. -F., Chen, H. -Y., Chen, C. -H., Chen, K. and Lee, K.H. (1994) *J. Nat. Prod.* **57**, 263.
278. Zeng, L., Gu, Z. -M., Fang, X. -P. and McLaughlin, J. L. (1994) *J. Nat. Prod.* **57**, 376.
279. Colman-Saizarbitoria, T., Zambrano, J., Ferrigni, N. R., Gu, Z. -M., Ng, J. H., Smith, D. L. and McLaughlin, J. L. (1994) *J. Nat. Prod.* **57**, 486.
280. Heltzel, C. E., Gunatilaka, A. A. L., Kingston, D. G. I., Hofmann, G. A. and Johnson, R. K. (1994) *J. Nat. Prod.* **57**, 620.
281. Bryant, F. O., Cutler, H. G., Parker, S. R. and Jacyno, J. M. (1994) *J. Nat. Prod.* **57**, 640.
282. Fullas, F., Hussain, R. A., Chai, H. -B., Pezzuto, J. M., Soejarto, D. D. and Kinghorn, A. D. (1994) *J. Nat. Prod.* **57**, 801.
283. Wu, T. -S., Ou, L. -F, and Teng, C. -M. (1994) *Phytochemistry* **36**, 1063.
284. Parmar, V. S. Bisht, K. S., Sharma, S. K., Jain, R., Taneja, P., Singh, S., Simonsen, O. and Boll, P. M. (1994) *Phytochemistry* **36**, 507.
285. Kazmi, M. H., Malik, A., Hameed, S., Akhtar, N. and Noor Ali, S. (1994) *Phytochemistry* **36**, 761.
286. Gören, N., Tahtasakal, E., Pezzuto, J. M., Cordell, G. A., Schwarz, B. and Proksch, P. (1994) *Phytochemistry* **36**, 389.
287. Zhou, B. -N., Bai, N. -S., Lin, L. -Z. and Cordell, G. A. (1994) *Phytochemistry* **36**, 721.
288. Ma, X., Lee, I. -S., Chai, H. -B., Zaw, K., Far-

nsworth, N. R., Soejarto, D. D., Cordell, G. A., Pezzuto, J. M. and Kinghorn, A. D. (1994) *Phytochemistry* **37**, 1659
289. Hou, R. -S., Duh, C. -Y., Wang, S. -K. and Chang, T. -T. (1994) *Phytochemistry* **35**, 271.
290. Hu, C. -Q. Chen, K., Shi, Q., Kilkuskie, R. E., Cheng, Y. -C. and Lee, K. -H. (1994) *J. Nat. Prod.* **57**, 42.
291. Wang, J. -N., Hou, C. -Y., Liu, Y. -L., Lin, L. -Z., Gil, RR. and Cordell, G. A. (1994) *J. Nat. Prod.* **57**, 211.
292. Fujioka, T., Kashiwada, Y., Kilkuskie, R. E., Cosentino, M., Ballas, L. M., Jiang, J. B., Janzen, W. P., Chen, I. S. and Lee, K. -H. (1994) *J. Nat. Prod.* **57**, 243.
293. Pengsuparp, T., Cai, L., Fong, H. H. S., Kinghorn, A. D., Pezzuto, J. M., Wani, M. C. and Wall M. E. (1994) *J. Nat. Prod.* **57**, 415.
294. Piacente, S., Aquino, R., de Tommasi, N., Pizza, C., Lock de Ugaz, O., Chavez Orellana, H. and Mahmood, N. (1994) *Phytochemistry* **36**, 991.
295. Burkhardt, G., Becker, H., Grubert, M., Thaomas, J and Eicher, T. (1994) *Phytochemistry* **37**, 1593.
296. García-Granados, A., Jiménez, M. B., Martínez, A., Parra, A., Rivas, F. and Arias, J. M. (1994) *Phytochemistry* **37**, 741.
297. Liu, J. -S. and Huang, M. -F. (1994) *Phytochemistry* **37**, 1759.
298. Sharma, M. C., Ohira, T. and Yatagi, M. (1994) *Phytochemistry* **37**, 201.
299. Greger, H., Hofer, O., Zechner, G., Hadacek, F. and Wurz, G (1994) *Phytochemistry* **37**, 1305.
300. Hirai, N., Ishida, H. and Koshimizu, K. (1994) *Phytochemistry* **37**, 383.
301. Nkengfack, A. E., Vouffo, T. W., Fomum, Z. T., Meyer, M., Bergendorff, O. and Sterner, O. (1994) *Phytochemistry* **36**, 1047.
302. Tahara, S., Katagiri, Y., Ingham, J. L. and Mizutani, J. (1994) *Phytochemistry* **36**, 1261.
303. Pinto, D. C. G., Fuzzati, N., Chiriboga Pazmino, X. and Hostettmann, K. (1994) *Phytochemistry* **37**, 875.
304. Simpol, L. R., Otsuka, H., Ohtani, K., Kasai, R. and Yamasaki, K. (1994) *Phytochemistry* **36**, 91.
305. Hsu, F. -L., Lee, Y. -Y. and Cheng, J. -T. (1994) *J. Nat. Prod.* **57**, 308.
306. Akihisa, T., Yasukawa, K., Kimura, Y., Takido, M., Kokke, W. C. M. C. and Tamura, T. (1994) *Phytochemistry* **36**, 153.
307. François, G., Bringmann, G., Phillipson, J. D., Aké Assi, L., Dochez, C., Rübenacker, M., Schneider, C., Wéry, M., Warhurst, D. C. and Kirby, G. C. (1994) *Phytochemistry* **35**, 1461.
308. Batista, O., Duarte, A., Nascimento, J., Simões, M. F., de la Torre, M. C. and Rodríguez, B. (1994) *J. Nat. Prod.* **57**, 858.
309. Dellar, J. E., Cole, M. D., Gray, A. I., Gibbons, S. and Waterman, P. G. (1994) *Phytochemistry* **36**, 957.
310. Ulubelen, A., Topçu, G., Eriş, C., Sönmez, U., Kartal, M., Kurucu, S. and Bozok-Johansson, C. (1994) *Phytochemistry* **36**, 971.
311. Osawa, K., Yasuda, H., Maruyama, T., Morita, H., Takeya, K. and Itokawa, H. (1994) *Phytochemistry* **36**, 1287.
312. El-Sebakhy, N., Asaad, A. M., Abdallah, R. M., Toaima, S. M., Abdel-Kader, M. S. and Stermitz, F. R. (1994) *Phytochemistry* **36**, 1387.
313. Matsuo, T., Hanamure, N., Shimoi, K., Nakamura, Y. and Tomita, I. (1994) *Phytochemistry* **36**, 1027.
314. Wu, T. -S., Leu, Y. -L., Chan, Y. -Y., Yu, S. -M., Teng, C. -M. and Su, J. -D. (1994) *Phytochemistry* **36**, 785.
315. Hidalgo, M. E., Fernández, E., Quilhot and Lissi, E. (1994) *Phytochemistry* **37**, 1585.
316. Minami, H., Kinoshita, M., Fukuyama, Y., Kodama, M., Yoshizawa, T., Sugiura, M., Nakagawa, K. and Tago, H. (1994) *Phytochemistry* **36**, 501.
317. Denyer, C. V., Jackson, P., Loakis, D. M., Ellis, M. R. and Young, D. A. B. (1994) *J. Nat. Prod.* **57**, 658.
318. Ueda, S., Umemura, T., Dohguchi, K., Matsuzaki, T., Tokuda, H., Nishino, H. and Iwashima, A. (1994) *Phytochemistry* **36**, 323.
319. Nakamura, O., Mimaki, Y., Nishino, H. and Sashida, Y. (1994) *Phytochemistry* **36**, 463.
320. Tada, M., Okuno, K., Chiba, K., Ohnishi, E. and Yoshii, T. (1994) *Phytochemistry* **35**, 539.
321. Russell, G. B., Hunt, M. B., Bowers, W. S. and Blunt, J. W. (1994) *Phytochemistry* **35**, 1455.
322. Orjala, J., Wright, A. J., Behrends, H., Folkers, G., Sticher, O., Rüegger, H., and Rali, T. (1994) *J. Nat. Prod.* **57**, 18.
323. Nick, A., Wright, A. D., Sticher, O. and Rali, T. (1994) *J. Nat. Prod.* **57**, 1245.
324. Collado, I. G., Aleu, J., Macías-Sánchez, Hernández-Galán, R. (1994) *J. Nat. Prod.* **57**, 738.
325. Grech, J. N., Li, Q., Roufogalis, B. D. and Duke, C. C. (1994) *J. Nat. Prod.* **57**, 1682.
326. Musza, L. L., Speight, P., McElhiney, S., Barrow, C. J., Gillum, A. M., Cooper, R. and Killar, L. M. (1994) *J. Nat. Prod.* **57**, 1498.
327. Haji, A., Momose, Y., Takeda, R., Nakanishi, S., Horiuchi, T. and Arisawa, M. (1994) *J. Nat. Prod.* **57**, 387.
328. Guardia, T., Guzman, J. A., Pestchanker, M. J., Guerreiro, E. and Giordano, O. S. (1994) *J. Nat. Prod.* **57**, 507.
329. Harrigan, G. G., Gunatilaka, A. A. L., Kingston, D. G. I., Chan, G. W. and Johnson, R. K. (1994) *J. Nat. Prod.* **57**, 68.
330. Gunatilaka, A. A. L., Kingston, D. G. I., Wijeratne, E. M. K., Bandara, B. M. R., Hofmann, G. A. and Johnson, R. K. (1994) *J. Nat. Prod.* **57**, 518.
331. Costa, S. S., Jossang, A., Bodo, B., Souza, M. L. M and Moraes, V. L. G. (1994) *J. Nat. Prod.* **57**, 1503.
332. Suksamararn, Eiamong, S., Piyachaturawat, P. and Charoenpiboonsin, J. (1994) *Phytochemistry* **36**, 1505.
333. Okada, N., Shirata, K., Niwano, M., Koshino, H. and Uramoto, M. (1994) *Phytochemistry* **37**, 281.

334. Veitch, N. C., Simmonds, M. S. J., Blaney, W. M. and Reymolds, T. (1994) *Phytochemistry* **35**, 1163.
335 Nakatani, M., Huang, R. -C., Okamura, H., Naoki, H. and Iwagawa, T. (1994) *Phytochemistry* **36**, 39.
336. Kumar, V., Bulumulla, H. N. K., Wimalasiri, W. R. and Reisch, J. (1994) *Phytochemistry* **36**, 879.
337. Miyazawa, M., Ishikawa, Y., Kasahara, H., Yamanaka, J. -I. and Kameoka, H. (1994) *Phytochemistry* **35**, 611.
338. González-Coloma, A., Escoubas, P., Mizutani, J. and Lajde, L. (1994) *Phytochemistry* **35**, 607.
339. Shishido, K., Nakano, K., Wariishi, N., Tateichi, H., Omodani, T., Shibuya, M., Goto, K., Ono, Y and Takaishi, Y. (1994) *Phytochemistry* **35**, 731.
340. Elliger, C. A., Haddon, W. F., Harden, L., Waiss, A. C., Jr., Wong, R. Y. (1994) *J. Nat. Prod.* **57**, 348.
341. Sauvain, M., Dedet, J. -P., Kunesch, N. and Poisson, J. (1994) *J. Nat. Prod.* **57**, 403.
342. Mahiou, V., Roblot, F., Hocquemiller, R., Cavé, A., Rojas de Arias, A., Inchausti, A., Yaluff, G., Fournet, A. and Angelo, A. (1994) *J. Nat. Prod.* **57**, 890.
343. Coates, N. J., Gilpin, M. L., Gwynn, M. N., Lewis, D. E., Milner, P. H., Spear, S. R. and Tyler, J. W. (1994) *J. Nat. Prod.* **57**, 654.
344. Matsunaga, K., Ikeda, M., Shibuya, M., and Ohizumi, Y. (1994) *J. Nat. Prod.* **57**, 1290.
345. Shobha, S. V., Ramadoss, C. S. and Ravidranath, B. (1994) *J. Nat. Prod.* **57**, 1755.
346. Thilborg, S. T., Christensen, S. B., Cornett, C., Olsen, C. E. and Lemmich, E. (1994) *Phytochemistry* **36**, 753.
347. Rocha, L., Marston, A., Kaplan, M. A. C., Stoeckli-Evans, H., Thull, U., Testa, B. and Hosetettmann, K. (1994) *Phytochemistry* **36**, 1381.
348. Nishida, R., Rothschild, M. and Mummery, R. (1994) *Phytochemistry* **36**, 37.
349. Kubo, I., Kinst-Hori, I. and Yokokawa, Y. (1994) *J. Nat. Prod.* **57**, 545.
350. Jurgens. T. M., Frazier, E. G., Schaeffer, J. M., Jones, T. E., Zink, D. L., Borris, R. P., Nanakorn, W., Beck, H. T. and Balick, M. J. (1994) *J. Nat. Prod.* **57**, 230.
351. Witherup, K. M., Bogusky, M. J., Anderson, P. S., Ramjit, H., Ransom, R. W., Wood, T. and Sardana, M. (1994) *J. Nat. Prod.* **57**, 1619.
352. Schmeller, T., Sauerwein, M., Sporer, F., Wink, M. and Müller, W. E. (1994) *J. Nat. Prod.* **57**, 1316.
353. Iinuma, M., Okawa, Y., Tanaka, T., Ho, F. -C., Kobayashi, Y. and Miyauchi, K. -I. (1994) *Phytochemistry* **37**, 889.
354. Blaakmeer, A., Stork, A., van Veldhuizen, A., van Beek, T. A., van Loon, J. J. A. and Schoonhoven, L. M. (1994) *J. Nat. Prod.* **57**, 90.
355. Nishida, R. (1994) *Phytochemistry* **36**, 873.
356. Brown, E. G. and Konuk, M. (1994) *Phytochemistry* **37**, 1589.
357. Fujita, T., Nishimura, H., Kaburagi, K. and Mizutani, J. (1994) *Phytochemistry* **36**, 23.
358. Baruah, N. C., Sarma, J. C., Barua, N. C., Sarma, S. and Sharma, R. P. (1994) *Phytochemistry* **36**, 29.
359. Kato-Noguchi, H. (1994) *Phytochemistry* **36**, 273.
360. Chung, M. -I., Jou, S. -J., Cheng, T. -H., Lin, C. -N., Ko, F. -N. and Teng, C. -M. (1994) *J. Nat. Prod.* **57**, 313.
361. Chen, I. -S., Wu, S. -J., Lin, Y. -C., Tsai, I. -L., Seki, H. Ko, F. -N. and Teng, C. -M. (1994) *Phytochemistry* **36**, 237.
362. Sheen, W. -S. Tsai, I. -L., Teng, C. -M. and I. -S. Chen (1994) *Phytochemistry* **36**, 213.
363. Chen, C. -C., Wu, L. -G., Ko, F. -N. and Teng, C. -M. (1994) *J. Nat. Prod.* **57**, 1271.
364. Achenbach, H., Hübner, H., Brandt, W. and Reiter, M. (1994) *Phytochemistry* **35**, 1527.
365. Kubo, I., Muroi, H. and Kubo, A. (1994) *J. Nat. Prod.* **57**, 9.
366. Chen, Q. -P., Deady, L. W. and Polya, G. M., (1994) *Phytochemistry* **36**, 1153.
367. Polya, G. M. and Foo, L. -Y. (1994) *Phytochemistry* **35**, 1399.
368. Zimmermann, M. I. and Sneden, A. T. (1994) *J. Nat. Prod.* **57**, 236.
369. Pathrina, C., Heymen, R. A. and Lazarchik, S. B. (1994) *J. Nat. Prod.* **57**, 1458.
370. Roddick, J. G. (1994) *Phytochemistry* **37**, 1277.
371. Matsunaga, K., Shibuya, M. and Ohizumi, Y. (1994) *J. Nat. Prod.* **57**, 1734.
372. Rao, R. C., Serradeil-Le Gal, C., Granger, I., Gleye, J., Augereau, J. -M., Bessibes, C. (1994) *J. Nat. Prod.* **57**, 1329.
373. You, M., Ma, X. -J., Mukherjee, R., Farnsworth, N. R., Cordell, G. A., Kinghorn, A. D. and J. M. Pezzuto (1994) *J. Nat. Prod.* **57**, 1517.
374. Sévenet, T. and Pusset, J. (1995) in *The Alkaloids* (*Vol.* 48) (Cordell. G. A., ed), p. 1. Academic Press, San Diego, CA, (in press).
375. Webby, R. F. and Markham, K. R. (1994) *Phytochemistry* **36**, 1323.
376. Gluchoff-Fiasson, K., Fiasson, J. L. and Favre-Bonvin, J. (1994) *Phytochemistry* **37**, 1629.
377. Chen, J. -L., Proteau, P. J., Roberts, M. A., Gerwick, W. H., Slate, D. L. and Lee, R. H. (1994) *J. Nat. Prod.* **57**, 524.
378. Nishida, R., Rothschild, M. and Mummery, R. (1994) *Phytochemistry* **36**, 37.
379. Zheng, G. -Q., Ho, D. K., Elder, P. J., Stephens, R. E., Cottrell, C. E. and Cassady, J. M. (1994) **57**, 32.
380. Nagashima, F., Ishimaru, A. and Asakawa, Y. (1994) *Phytochemistry* **37**, 1767.
381. Weber, H. A. and Gloer, J. B. (1991) *J Org. Chem.* **53**, 4355 (and Refs therein).
382. Alfatafta, A. A., Gloer, J. B., Scott, J. A. and Malloch, D. (1994) *J. Nat. Prod.* **57**, 1696.
383. Keller, K. (1991) *J. Ethnopharmacol.* **32**, 225.
384. Anand, N., Bindra, J. S. and Ranganathan, S. (1988) *Art in Organic Synthesis* (2nd edn). Wiley Interscience, New York.
385. Corey, E. J. and Cheng, X. -M. (1989) *The Logic of Chemical Synthesis.* Wiley Interscience, New York.
386. Cornforth, J. W. (1993) *Aust. J. Chem.* **46**, 157.
387. Davies, S. G. (1989) *Chem. Brit.* 268.
388. Brown, J. M. (1989) *Chem. Brit.* 276.

389. Lawrence, N. J. (1994) *Ann. Repts. Sec. B* **90**, 269.
390. Fleet, G. W. (1989) *Chem. Brit.* 287.
391. Gordge, P. C., Darcy, P., A. T. Evans, Ryves, W. J., Evans, F. J. and Hassan, N. M. (1994) *Phytother. Res.* **8**, 362.
392. Walker, D. G., Swigor, J. E., Kant, J. and Schroeder, D. R. (1994) *J. Labelled Compound Radiopharmacol.* **34**, 973.
393. Seltzman, H. H., Odear, D. F., Carroll, F. I., and Wyrick, C. D. (1992) *J. Chem. Soc., Chem. Commun.* 1757.
394. Lotsoff, H. S. (1986) US Pat. 4,587,243.
395. Madras, B. K. and Meltzer, P. (1994) PCT Int. Appl. WO 94 04,146. *Chem. Abstr.* **121**, 134539h.
396. Neumeyer, J. L., Wang, S. -Y., Milius, R. A., Baldwin, R. M., Zea-Ponce, Y., Hoffer, P. B., Sybirska, E., Al-Tikriti, M., Charney, D. S., Malison, Laruelle, M. and Innis, R. B. (1991) *J. Med. Chem.* **34**, 3144.
397. Landry, D. W., Zhao, K., Yang, G. X. -Q., Glickman, M. and T. M. Georgiadis (1993) *Science* **259**, 1899.
398. Neidigh, K. A., Kingston, D. G. I. and Lewis, N. G. (1994) *J. Nat. Prod.* **57**, 791.
399. Vederas, J. C. (1987) *Nat. Prod. Rep.* **4**, 277.
400. Verpoorte, R., van der Heijden, R., Schripsema, J., Hoge, J. H. C. and ten Hoopen, H. J. G. (1993) *J. Nat. Prod.* **56**, 186.
401. Scott, A. I. (1993) *Pure Appl. Chem.* **65**, 1299.
402. Kutchan, T. M. (1993) *Phytochemistry* **32**, 493.
403. Donadio, S., Staver, M. J., McAlpine, J. B., Swanson, S.J. and Katz, L. (1991) *Science* **252**, 675.
404. Weber, J. M., Leung, Swanson, S. J., Idler, K. B. and McAlpine, J. B. (1991) *Science* **252**, 114.
405. McDaniel, R., Ebert-Khosala, S., Hopwood, D. A. and Khosala, C. (1994) *J. Am. Chem. Soc.* **116**, 10855 (and Ref therein).
406. Akashi, T., Fuuno, T., Takahashi, T. and Ayabe, S.-I. (1994) *Phytochemistry* **36**, 303.
407. Furuya, T., Ikuta, A. and Syono, K. (1972) *Phytochemistry* **11**, 3041.
408. Verpoorte, R., van der Heijden, R., van Gulik, W. M. and ten Hoopen, H. J. G. (1991) in *The Alkaloids* (Vol. 40) (Brossi, A., ed), p. 1. Academic Press, San Diego, CA.
409. Verpoorte, R., Schripsema, J. and van der Leer, (1988) in *The Alkaloids* (Vol. 34) (Brossi, A., ed). p. 332. Academic Press, New York.
410. Moore, B. S., Cho, H., Casati, R., Kennedy, E., Reynolds, K. A., Mocek, U., Beale, J. M. and Floss, H. G. (1993) *J. Am. Chem. Soc.* **115**, 5254.
411. Moore, B. S., Poralla, K. and Floss, H. G. (1993) *J. Am. Chem. Soc.* **115**, 5267.
412. Decendit, A., Liu, D., Ouelhazi, L., Doireau, P., Mérillon, J. M. and Rideau, M. (1992) *Plant Cell Rep.* **11**, 400.
413. Hara, M., Tanaka, S. and Tabata, M. (1994) *Phytochemistry* **36**, 327.
414. Bauer, W. and Zenk, M. H. (1989) *Tetrahedron Letters* **30**, 5257.
415. Rueffer, M. and Zenk, M. H. (1994) *Phytochemistry* **36**, 1219.
416. Keen, N. T., Partridge, J. E. and Zaki, A. I. (1972) *Phytopathology* **62**, 768.
417. Flores, H. E. (1992) *Chem. Ind.* 374.
418. Towers, G. H. N. and Ellis, S. (1993) *Human Medicinal Agents from Plants* (Kinghorn, A. D. and Balandrin, M. F., eds), p. 56. American Chemical, Society Symposium Series No. 534, Washington, DC.
419. Heinstein, P. (1985) *J. Nat. Prod.* **48**, 1.
420. Benjamin, B. D., Roja, G. and Heble, M. R. (1994) *Phytochemistry* **35**, 381.
421. Schumacher, H. -M., Gundlach, H., Fiedler, F. and Zenk, M. H. (1987). *Plant Cell Rep.* **6**, 410.
422. Kutchan, T. M. and Zenk, M. H. (1993) *J. Plant Res.* **3**, 165.
423. Eilert, U., Kurz, W. G. W. and Constabel, F. (1985) *J. Plant Physiol.* **119**, 65
424. Kammerer, L., De-Eknamkul, W. and Zenk, M. H. (1994) *Phytochemistry* **36**, 1409.
425. De-Eknamkul, W., Tanahashi, T. and Zenk, M. H. (1992) *Phytochemistry* **31**, 2713.
426. Mahady, G. B. and Beecher, C. W. W. (1994) *Phytochemistry* **37**, 415.
427. Roja, G. and Heble, M. R. (1994) *Phytochemistry* **36**, 65.
428. Brown, G. D. (1994) *J. Nat. Prod.* **57**, 975.
429. Montforte-Gonzalez, M., Ayora-Talavera, T., Maldonado-Mendoza, I. E., and Loyola-Varga, V. M. (1992) *Phytochem. Anal.* **3**, 117.
430. Kikuchi, Y., Irie, M., Yoshimatsu, K., Ishimaru, K., Shiomomura, K., Satake, M., Sueyoshi, S., Tanno, M., Kamiya, S., Sawada, J. -I. and Terao, T. (1991) *Phytochemistry* **30**, 3273.
431. Robins, R. J. and Walton, N. J. (1993) in *The Alkaloids* (Vol. 44). (Cordell, G. A., ed), p. 116. Academic Press, San Diego, CA.
432. Portsteffen, A., Dräger, B. and Nahrstedt, A. (1992) *Phytochemistry* **31**, 1135.
433. Portsteffen, A., Dräger, B. and Nahrstedt, A. (1994) *Phytochemistry* **37**, 391.
434. Hashimoto, T., Nakajima, K., Ongena, G. and Yamada, Y. (1992) *Plant Physiol.* **100**, 836.
435. Dräger, B. and Schaal, A. (1994) *Phytochemistry* **35**, 1441.
436. Leete, E. (1979) *Planta Med.* **36**, 97.
437. Leete, E. (1990) *Planta Med.* **56**, 339.
438. Leete, E. (1960) *J. Am. Chem. Soc.* **82**, 612.
439. Ansarin, M. and Woolley, J.G. (1993) *J. Nat. Prod.* **56**, 1211.
440. Ansarin, M. and Woolley, J. G. (1993) *Phytochemistry* **32**, 1183.
441. Underhill, E. W. and Youngken, H. W. (1962) *J. Pharm. Sci.* **51**, 121.
442. Leete, E., Kokwanko, N. and Newark, R.A. (1975) *J. Am. Chem. Soc.* **97**, 6826.
443. Ansarin, M. and Woolley, J. G. (1994) *Phytochemistry* **35**, 935.
444. Saito, K., Suzuki, H., Takamatsu, S. and Murakoshi, I. (1993) *Phytochemistry* **32**, 87.

445. Saito, K., Koike, Y., Suzuki, H. and Murakoshi, I. (1993) *Phytochemistry* **34**, 1041.
446. Saito, K., Suzuki, H., Yamashita, Y. and Murakoshi, I. (1994) *Phytochemistry* **36**, 309.
447. Ratcliffe, R.G. and Roberts, J. K. M. (1990) *Magn. Reson. Med. Biol.* **4**, 77.
448. Forad, Y. -Y., Fox, G. G., Ratliffe, R. G. and Robins, R. J. (1994) *Phytochemistry* **36**, 333.
449. Iiyama, K., Tuyet Lam, T. B., Kasuya, N. and Stone, B. A. (1994) *Phytochemistry* **35**, 959.
450. Bacon, J. S. D. and Gordon, A. H. (1980) *J. Agric. Sci. Camb.* **94**, 361.
451. Titgemeyer, E. C., Bourquin, L. D. and Fahey, G. C. Jr (1992) *J. Sci. Food Agric.* **58**, 451.
452. Scott, A. I. (1994) *J. Nat. Prod.* **57**, 557.
453. Spencer, J. B., Stolowich, N. J., Roessner, C. A., Min, C. and Scott, A. I. (1993) *J. Am. Chem. Soc.* **115**, 11610.
454. Min, C., Atshaves, B. P., Roessner, C. A., Stolowich, N. J., Spencer, J. B. and Scott, A. I. (1993) *J. Am. Chem. Soc.* **115**, 10380.
455. Hapiot, P., Pinson, J., Neta, P., Franesch, C., Mhamdi, F., Rolando, C. and Schneider, S. (1994) *Phytochemistry* **36**, 1013.
456. Mazzafera, P., Wingsle, G., Olffon, O. and Sandberg, G. (1994) *Phytochemistry* **37**, 1577.
457. Roberts, M. F. and Waller, G. R. (1979) *Phytochemistry* **18**, 451.
458. Cordell, G. A. (1981) in *Proceedings of the 4th Asian Symposium on Medicinal Plants and Spices*, p. 213. UNESCO, Bangkok, Thailand.
459. Heinstein, P. F. and Chang, C. J. (1994) *Ann. Rev. Plant Physiol. Plant Mol. Biol.* **45**, 663.
460. Kingston, D. G. I., Molinero, A. A. and Rimoldi, F. X. (1993) *Progr. Chem. Org. Naturst.* **61**, 1.
461. Cordell, G. A. (1974) *Lloydia* **37**, 219.
462. Zamir, L. O., Zhou, Z. H., Caron, G., Nedea, M. E., Sauriol, F. and Mamer, O. (1995) *J. Chem. Soc., Chem. Commun.* 529.
463. Zenk, M. H. (1991) *Phytochemistry* **30**, 3861.
464. Stöckigt, J. and Zenk, M. H. (1977) *J. Chem. Soc., Chem. Commun.* 646.
465. Kutchan, T. M., Hampp, N., Lottspeich, F., Beyreuther, K. and Zenk, M. H. (1988) *FEBS Lett.* **237**, 40.
466. Steffens, P., Nagakura, N. and Zenk, M. H. (1985) *Phytochemistry* **24**, 2577.
467. Dittrich, H. and Kutchan, T. M. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 9969.
468. Kutchan, T. M., Bock, A. and Dittrich, H. (1994) *Phytochemistry* **35**, 353.