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Introduction

Over the millennia, humans have relied on nature for their basic needs, and not least as a source of medicines. Plants have formed the basis of traditional medical systems that have been in existence for thousands of years, and the first records, written on clay tablets in cuneiform, are from Mesopotamia and date from about 2600 BCE. Egyptian medicine dates from about 2900 BCE, with the best known Egyptian pharmaceutical record being the Ebers Papyrus dating from 1500 BCE. The Chinese Materia Medica has been extensively documented over the centuries, with the first record (Wu Shi Er Bing Fang), containing 52 prescriptions, dating from about 1100 BCE, though records from the Pent’sao are reputed to be even earlier (~2700 BCE); documentation of the Indian Ayurvedic system dates from about 1000 BCE (Susruta and Charaka). The use of plants in the traditional medicine systems of many other cultures, including the Maya (Berlin and Berlin, 1996), has been documented, and Arvigo and Balick have recorded the use of a subset of plants for medicinal purposes in Belize (Arvigo and Balick, 1993).

These plant-based systems continue to play an essential role in healthcare, and the World Health Organization has estimated that approximately 80% of the world’s inhabitants rely mainly on traditional medicines for their primary health care. In addition, plant products also play an important role in the health care systems of the remaining 20% of the population, mainly residing in industrialized countries (Farnsworth et al., 1985). Indeed, molecules derived from natural
sources (so-called natural products), including plants, marine organisms and microorganisms, have played, and continue to play, a dominant role in the discovery of leads for the development of conventional drugs for the treatment of most human diseases (Newman and Cragg, 2007).

Despite the intensive investigation of terrestrial flora, it is estimated that only 6% of the approximately 300,000 species of higher plants have been systematically investigated, pharmacologically, and only some 15% phytochemically (Balandrin et al., 1993). The medicinal potential of other realms of nature such as marine organisms and microorganisms has barely been tapped. A country such as Belize which is rich in plant and other biological resources, and which has a history and ongoing record in the beneficial use of plants in traditional medicine, offers promising potential for the discovery of novel drugs for the treatment of a variety of diseases.

Thus in 1987, The New York Botanical Garden, through a contract with the United States National Cancer Institute (NCI) for plant collections in Central and South America, established a collaboration with local experts in Belize for the collection of plants as a source of potential anti-cancer and anti-HIV agents. This chapter provides information on the natural products research carried out by the NCI from its early stages to the present, including the work in Belize, along with information on NCI’s benefit sharing program developed to compensate source countries for their collaboration in this global research effort.

**Drug Discovery and Development at the NCI, 1960-1982**

The United States National Cancer Institute (NCI) was established in 1937, its mission being “to provide for, foster and aid in coordinating research related to cancer.” In 1955, NCI set up the Cancer Chemotherapy National Service Center (CCNSC) to coordinate a national
voluntary cooperative cancer chemotherapy program, involving the procurement of drugs, screening, preclinical studies, and clinical evaluation of new agents. By 1958 the initial service nature of the organization had evolved into a drug research and development program with input from academic sources and substantial participation of the pharmaceutical industry. The responsibility for drug discovery and preclinical development at NCI now rests with the Developmental Therapeutics Program (DTP), a major component of the Division of Cancer Treatment, Diagnosis and Centers (DCTDC). Thus, NCI has, for the past forty years, provided a resource for the preclinical screening of compounds and materials submitted by grantees, contractors, pharmaceutical and chemical companies, and other scientists and institutions, public and private, worldwide, and has played a major role in the discovery and development of many of the available commercial and investigational anticancer agents (Cragg and Newman, 2006). During this period, more than 400,000 chemicals, both synthetic and natural, have been screened for antitumor activity.

Initially, most of the materials screened were pure compounds of synthetic origin, but the program also recognized that natural products were an excellent source of complex chemicals with a wide variety of biological activities. During the early years of the CCNSC, the screening of natural products was concerned mainly with the testing of microbial fermentation products, and, prior to 1960, only about 1,500 plant extracts were screened for antitumor activity. Earlier work on the isolation of active antitumor agents from *Podophyllum peltatum* L., the Mayapple found throughout the eastern U.S. and used by early American cultures for the treatment of skin lesions and warts (Hartwell, 1982; Jardine, 1979), and the discovery and development of vinblastine and vincristine, used in the treatment of childhood leukemia and other cancers, from the rosy periwinkle, *Catharanthus roseus* (L.) G. Don (Johnson, Wright, and Svoboda, 1959;
Carter and Livingston 1976), however, provided convincing evidence that plants could be sources of a variety of novel potential cancer chemotherapeutic agents. A decision was made to explore plants more extensively as sources of agents with antitumor activity, and, in 1960, an interagency agreement was established with the United States Department of Agriculture (USDA) for the collection of plants for screening in the CCNSC program. A small number of animal extracts, mainly of marine origin, were also tested beginning in 1960, but by the end of 1968, only 1,000 animal extracts had been screened. The pace of investigation of marine invertebrates accelerated in the 1970s and, by 1982, over 16,000 extracts had been screened. In contrast, however, from 1960 to 1982, over 180,000 microbial fermentation products and over 114,000 plant-derived extracts were tested for in vivo antitumor activity, mainly using the L1210 and P388 mouse leukemia models. Extracts showing significant activity were subjected to bioassay-guided fractionation, and the isolated active agents were submitted for secondary testing against panels comprising four to eight animal tumor models and human tumor xenografts (Driscoll, 1984; Venditti, Wesley, and Plowman, 1984). Those agents showing significant activity in the secondary panel were assigned priorities for preclinical and clinical development.

Much of the drug-discovery effort was carried out through collaborations with research organizations and the pharmaceutical industry, which either submitted compounds on a voluntary basis or were supported by NCI through contract or grant-funding mechanisms. Of 155 commercial and approved anticancer drugs currently available, 64% may be classified as being of natural origin or naturally inspired (Newman and Cragg, 2007). While the majority of these drugs were discovered outside the NCI program, the NCI did play a significant role in the development of many of them.
Anticancer Agents Derived From Plant Sources

Plants have a long history of use in the treatment of cancer (Hartwell, 1982), though many of the claims for the efficacy of such treatment should be viewed with some skepticism because cancer, as a specific disease entity, is likely to be poorly defined in terms of folklore and traditional medicine (Cragg and Newman, 2005). Of the plant-derived anticancer drugs in clinical use, the best known are the so-called vinca alkaloids, vinblastine and vincristine, isolated from the Madagascar periwinkle, *Catharanthus roseus*. *Catharanthus roseus* was used by various cultures for the treatment of diabetes, and vinblastine and vincristine were first discovered during an investigation of the plant as a source of potential oral hypoglycemic agents. Therefore, their discovery may be indirectly attributed to the observation of an unrelated medicinal use of the source plant (Cragg and Newman 2005). Two further drugs derived from vinblastine through semi-synthetic modifications are vinorelbine and vindesine. The two clinically-active agents, etoposide and teniposide, which are semi-synthetic derivatives of the natural product epipodophyllotoxin, may be considered being more closely linked to a plant originally used for the treatment of “cancer”. Epipodophyllotoxin is an isomer of podophyllotoxin, which was isolated as the active antitumor agent from the roots of various species of the genus *Podophyllum*. These plants possess a long history of medicinal use by early American and Asian cultures, including the treatment of skin cancers and warts (Cragg and Newman, 2005).

More recent additions to the armamentarium of naturally-derived chemotherapeutic agents are the taxanes and camptothecins. Paclitaxel initially was isolated from the bark of *Taxus brevifolia* Nutt., collected in Washington State as part of a random collection program by the
U.S. Department of Agriculture for the National Cancer Institute [NCI] (Cragg and Newman, 2004). The use of various parts of *T. brevifolia* and other *Taxus* species (e.g., *T. canadensis* Marsh., *T. baccata* L.) by several Native American tribes for the treatment of some non-cancerous conditions has been reported (Cragg *et al.*, 1993a), while the leaves of *T. baccata* are used in the traditional Asiatic Indian (Ayurvedic) medicine system, with one reported use in the treatment of “cancer” (Hartwell, 1982). Paclitaxel, along with several key precursors (the baccatins), occurs in the leaves of various *Taxus* species, and the ready semi-synthetic conversion of the relatively abundant baccatins to paclitaxel, as well as active paclitaxel analogs, such as docetaxel) (Cragg and Newman, 2004), has provided a major, renewable natural source of this important class of drugs. Likewise, the clinically active agents, topotecan (hycamptamine), irinotecan (CPT-11) and 9-aminocamptothecin, are semi-synthetically derived from camptothecin, isolated from the Chinese ornamental tree, *Camptotheca acuminata* Decne. (Cragg and Newman, 2004). Camptothecin (as its sodium salt) was advanced to clinical trials by NCI in the 1970s, but was dropped because of severe bladder toxicity.

Other examples of plant-derived agents currently in investigational use are homoharringtonine, isolated from the Chinese tree, *Cephalotaxus harringtonia* var. *drupacea* (Sieb and Zucc.) Koidz., and elliptinium, a derivative of ellipticine, isolated from species of several genera of the Apocynaceae family, including *Bleekeria vitensis* A. C. Sm., a Fijian medicinal plant with reputed anticancer properties (Cragg and Newman, 2005). Homoharringtonine has shown efficacy against various leukemias, while elliptinium is marketed in France for the treatment of breast cancer (Suffness and Cordell, 1985). The flavone, flavopiridol (16), is currently in Phase III clinical trials, having been resuscitated by its potential as a treatment for chronic myelogenous leukemia (CML) by workers at Ohio State University.
While flavopiridol is totally synthetic, the basis for its novel structure is a natural product, rohitukine, isolated from *Dysoxylum binectariferum* Hook. F. ex Bedd.

A number of other plant-derived agents were entered into clinical trials and were terminated due to lack of efficacy or unacceptable toxicity. Some examples are acronycine, bruceantin, maytansine and thalicarpine (Cragg and Newman, 2005).

**Primary Antitumor Screening at the NCI: Current Status**

A given organism such as a plant provides the investigator with a complex library of unique bioactive chemical constituents. The task of the natural products researcher is to select those compounds of pharmacological interest through bioassay-guided fractionation of the “natural chemical libraries” produced by extraction of the organisms, and then to collaborate in the optimization and development of the so-called lead natural product structure to provide an effective drug. The successful development of effective new drugs requires suitable assays to guide not only the discovery of a bioactive lead, but also the evaluation of analogues developed through optimization of the lead.

**Molecular Target Assays**

Natural products often possess highly selective and specific biological activities. In the early days of natural products research, new compounds were simply isolated at random, or at best, by the use of simple broad-based bioactivity screens based on antimicrobial or cytotoxic activities. Although these screens did result in the isolation of many bioactive compounds (Newman *et al.*, 2000), nowadays they are not considered sufficiently specific for the discovery of the next generation of drugs. A large number of specific biochemical and genetics-based
screens using transformed cells, a key regulatory intermediate in a biochemical or genetic pathway, or a receptor-ligand interaction have been developed, and are now in routine use. These screens permit the more exact detection of bioactive compounds in the complex chemical mixtures that are natural product extracts, and provide important preliminary data about the mechanism of action (MOA) of the isolated compounds early in the discovery process. This preliminary knowledge of how a compound may interfere with the progress of a disease process can be valuable in determining whether or not to prioritize the compound for further development.

These new biochemical and genetic screens are highly automated, high throughput assays (upwards of 50,000 assay points per day in a number of cases). They permit the rapid testing of large libraries (hundreds of thousands) of pure compounds or natural products extracts, and are being employed as targets for natural product lead discovery (Klekota et al., 2006). Since screening capacity is no longer the rate-limiting step, many major drug-discovery groups including academia and industry are becoming very interested in screening natural products (either as crude extracts or as prefractionated “peak libraries”) as a low-cost means of discovering novel lead compounds.

**Cell-based Assays**

While some of the molecular target screens alluded to above may involve use of transformed cells, the NCI’s 60 cell-line cytotoxicity screen for antitumor agents represents a more traditional cell-based screen. It has been described in detail (Boyd and Paull, 1995) and, although this is not a true receptor-based screen, it has now been developed into a system whereby a large number of molecular-targets within the cell lines may be identified by informatics techniques, and refinements are continuing. Information as to the current status of
the screens involved is available at the URL http://dtp.nci.nih.gov/screening.html. In addition, there are simple but robust assays that can be used by workers in academia who do not have access to, or may not need, high throughput screens. Examples are the brine shrimp and potato disc assays (McLaughlin, 1991; Meyer et al., 1982)

**In vivo Assays**

Natural products identified from molecular target or traditional cell-based assays are further evaluated for their antitumor potential using *in vivo* assays. The NCI uses the Hollow Fiber Assay (Hollingshead *et al.*, 1995a) as a relatively inexpensive *in vivo* prescreen to prioritize compounds for testing in the more definitive human tumor xenograft models (Alley *et al.*, 2004). Currently, both the Hollow Fiber Assay and xenograft models are being evaluated for the prioritization of natural product extracts for fractionation. Information on the regular NCI *in vivo* assays, including a detailed description of the protocol used in the Hollow Fiber Assay, is available at the URL http://dtp.nci.nih.gov/screening.html.

**Anti-HIV Screening**

As part of the response of the National Institutes of Health (NIH) to the AIDS epidemic, in 1987 the Developmental Therapeutics Program (DTP) of the NCI developed a screening program for the large-scale testing of synthetic and natural products for anti-HIV activity (Boyd, 1988). Briefly, the anti-HIV screening assay (Weislow *et al.*, 1989) used human lymphoblastoid cells (CEM-SS cells) which were grown in the presence or absence of the human immunodeficiency virus (HIV-1), and in the presence or absence of test material. Anti-HIV activity was indicated by an enhanced growth or survival of the virus-infected cells in the
presence of the test material. This cell-based screen has been replaced by a series of mechanism-based assays.

**Advanced Preclinical Development**

Those agents showing significant *in vivo* activity are evaluated by appropriate NCI committees, and, if approved, they are entered into advanced preclinical development. The steps in the preclinical development process involve:

- The procurement of an adequate supply of any natural product drug to permit preclinical and clinical development.
- Formulation studies to develop a suitable vehicle to solubilize the drug to enable administration to patients, generally by intravenous injection or infusion in the case of cancer. The low solubility of many natural products in water poses considerable problems, but these can be overcome by use of cosolvents or emulsifying agents (surfactants) such as Cremophore EL® (polyoxyethylated castor oil).
- Pharmacological evaluation to determine the best route and schedule of administration to achieve optimal activity of the drug in suitable animal models, the determination of the half-lives and bioavailability of the drug in blood and plasma, the rates of clearance and the routes of excretion, and the identity and rates of formation of possible metabolites.
• The final step involving toxicological studies to determine the type and degree of major toxicities in rodent and dog models. These studies help to establish the safe starting doses for administration to human patients in clinical trials.

Clinical development

Those agents having acceptable pharmacological and toxicological properties (Section 6) enter Phase I studies to determine the maximum tolerated dose (MTD) of a drug in humans, and to observe the sites and reversibilities of any toxic effects. Once the MTD has been determined and the clinicians are satisfied that no insurmountable problems exist with toxicities, the drug advances to Phase II clinical trials. These trials are generally conducted to test the efficacy of the drug against a range of different cancer disease types. In those cancers where significant responses are observed, Phase III trials are conducted to compare the activity of the drug with that of the best chemotherapeutic agents currently available for the treatment of those cancers. In addition, the new drug may be tried in combination with other effective agents to determine if the efficacy of the combined regimen exceeds that of the individual drugs used alone.

For information on the development of some recently discovered anti-cancer agents, interested readers are referred to the review by Cragg and Newman (2006).

Drug Discovery and Development at the NCI: Current Status

As discussed earlier, from 1960 to 1982 a considerable number of natural product extracts, particularly of microbial and plant origin, were screened for antitumor activity, and a number of clinically effective chemotherapeutic agents were developed. In the early 1980s, however, this program was discontinued since it was perceived that few novel active leads were
being isolated from natural sources. This conclusion applied equally to plants, marine organisms and microorganisms, and resulted in a general de-emphasis of natural products as potential sources of novel antitumor agents. Of particular concern was the failure to identify agents possessing activity against the resistant solid tumor disease-types. This apparent failure might however, be attributed more to the nature of the primary screens being used at the time, rather than to a deficiency of Nature. Continued use of the primary P388 mouse leukemia screen, which was a fast-growing tumor line, appeared to be detecting only known active compounds or chemical structure types having little or no activity against solid tumors, which are inherently slow-growing lines.

The revision of the antitumor screening strategy in 1985, and the development of the new \textit{in vitro} human cancer cell line screen led to the implementation of a new NCI natural products program involving new procurement, extraction and isolation components. The initiation, in 1987, of a major new program within the NCI for the discovery and development of anti-HIV agents provided yet further impetus and resources for the revitalization of the NCI’s focus upon natural products. Contracts for the cultivation and extraction of fungi and cyanobacteria, and for the collection of marine invertebrates and plants, were initiated in 1986 and, with the exception of fungi and cyanobacteria, these programs are continuing to operate, albeit at reduced overall collection levels due to budgetary constraints. Marine organism collections had focused on the Southwestern Pacific Ocean, but have now expanded worldwide through a contract with the Coral Reef Research Foundation. Terrestrial plant collections have been carried out in over 25 countries in tropical and subtropical regions worldwide through contracts with Missouri Botanical Garden (Africa and Madagascar), The New York Botanical Garden (Central and South America and the Caribbean), and the University of Illinois at Chicago (Southeast Asia). Since
1996, the contract collection program in Central and South America has been replaced by collaborations with organizations in several source countries, while collections in Africa and Southeast Asia are continuing on a reduced scale through purchase order-based operations with the same organizations. Collections in the continental United States were started in 1996 through a contract with the Morton Arboretum, and later, World Botanical Associates.

In carrying out these collections, the NCI contractors work closely with qualified organizations in each of the source countries. To date, botanists and marine biologists from source country organizations have collaborated in field collection activities and taxonomic identifications, and their knowledge of local species and conditions has been indispensable to the success of the NCI collection operations. Source country organizations provide facilities for the preparation, packaging, and shipment of the samples to the NCI natural products repository in Frederick, Maryland. The collaboration between the source country organizations and the NCI collection contractors has, in turn, provided support for expanded research activities by source country biologists, and the deposition of a voucher specimen of each species collected in the national herbarium or repository is expanding source country holdings of their biota. When requested, NCI contractors also provide training opportunities for local personnel through conducting workshops and presentation of lectures. In addition, through its Letter of Collection (LOC) and agreements based upon it, the NCI invites scientists (as funding permits) nominated by Source Country Organizations to visit its facilities, or equivalent facilities in other approved U.S. organizations for 3-12 months to participate in collaborative natural products research, while representatives of most of the source countries have visited the NCI and contractor facilities for shorter periods to discuss collaboration.
Dried plant samples (0.3-1 kg dry weight) and frozen marine organism samples (~ 1 kg wet weight) are shipped to the NCI Natural Products Repository (NPR) in Frederick where they are stored at -20°C prior to extraction with a 1:1 mixture of methanol: dichloro-methane and water to give organic solvent and aqueous extracts. All the extracts are assigned discrete NCI extract numbers and returned to the NPR for storage at -20°C until requested for screening or further investigation (http://dtp.nci.nih.gov/branches/npb/repository.html). After testing in the in vitro human cancer cell line and the anti-HIV screens, active extracts are subjected to bioassay-guided fractionation to isolate and characterize the pure, active constituents. Agents showing significant activity in the primary in vitro screens are selected for secondary testing. The in vivo activity of potential anticancer agents is first assessed using a hollow fiber encapsulation/implantation methodology (Section 4.3), and those agents exhibiting significant activity are then assessed against athymic mouse xenografts which reflect the complex phenomena occurring when the tumor cells are growing in and interacting with the host’s normal tissues (e.g. angiogenesis effects, metastatic potential). In the case of potential anti-AIDS drugs, the hollow fiber methodology using HIV-infected cells was used to assess efficacy (Hollingshead et al., 1995b).

Source Country Collaboration and Compensation

The recognition of the value of the natural resources (plant, marine and microbial) being investigated by the NCI, and the significant contributions being made by source country scientists and traditional healers in aiding the performance of the NCI collection programs, have led the NCI to formulate policies aimed at facilitating collaboration with, and compensation of, countries participating in the NCI drug discovery program. Many of these countries are
developing nations, which have a real sensitivity to the possibility of exploitation of their natural resources by developed country organizations involved in drug discovery or other programs searching for novel bioactive agents.

The Letter of Collection formulated by the NCI (http://ttc.nci.nih.gov/forms; Appendix 1) contains both short- and long-term measures aimed at assuring countries participating in NCI-funded collections of its intentions to deal with them in a fair and equitable manner. In the short-term, the NCI periodically invites scientists from local organizations to visit the drug discovery facilities at the NCI Frederick to discuss the goals of the program, and to explore the scope of collaboration in the drug discovery effort. When laboratory space and resources permit, suitably qualified scientists are invited to spend periods of up to 12 months working with scientists in NCI or other suitable facilities on projects related to natural products drug discovery, such as the testing of extracts and the bioassay-guided isolation and structure determination of active agents, preferably from organisms collected in their home countries.

As test data become available from the anti-cancer and anti-HIV screens, these are provided to the NCI collection contractors for dissemination to interested scientists in countries participating in the NCI collection programs. Each country receives only data obtained from extracts of organisms collected within their borders, and scientists are requested to keep data on active organisms confidential until the NCI has had sufficient time to assess the potential for the development of novel drugs from such organisms. Confidentiality is an important issue, since the NCI will apply for patents on agents isolated in its laboratories by NCI scientists showing particular promise; such patents may be licensed to pharmaceutical companies for development and eventual marketing of the drugs. As part of the licensing agreement, the NCI requires the successful licensee to negotiate and enter into agreement(s) with an appropriate organization(s)
in the country of origin of the organism yielding the drug. Such an agreement will address the concern on the part of the source country that pertinent agencies, institutions and/or persons or communities receive royalties and other forms of compensation, as appropriate. Such agreements apply equally to instances where the drug is structurally based on the isolated natural product, though the percentage of royalties or compensation may vary depending on the relationship of the marketed drug to the originally isolated product. Such compensation is regarded as a potential long-term benefit, since development of a drug to the stage of marketing can take from 10 to 20 years from its time of discovery.

Another potential benefit to the country of origin is the development of a large-scale cultivation program to supply sufficient raw material for bulk production of the drug. In licensing a patent on a new drug to a pharmaceutical company, the NCI will require the licensee to seek, as its first source of supply, the raw material produced in the country of origin. The policies concerning royalty payments and raw material supplies will also apply to inventions made by other organizations screening NCI extracts for activities against diseases related to the NCI mission. In addition, organizations investigating the large-scale production of biomass for the isolation of drugs of interest to NCI will be required to explore collaboration with the relevant source countries in the performance of their tasks.

The discovery and development of the calanolides, isolated from the Sarawak (Malaysia) plants, *Calophyllum lanigerum* Miq. and *C. teysmannii* Miq. (Kashman et al., 1992) illustrates the potential for international collaboration resulting from the contract collection program supported by the NCI. An extract of the leaves and twigs of the tree, *Calophyllum lanigerum*, collected in Sarawak, Malaysia in 1987, yielded (+)-calanolide A which showed significant anti-HIV activity. Efforts to relocate the original tree failed, and collections of other specimens of the
same species gave only trace amounts of calanolide A. A detailed survey of *C. lanigerum* and related species showed that latex of *Calophyllum teysmannii* yielded extracts with significant anti-HIV activity. The active constituent was found to be (-)-calanolide B (Fig. 2), which was isolated in yields of 20 to 30%. While (-)-calanolide B is slightly less active than (+)-calanolide A, it has the advantage of being readily available from the latex which is tapped in a sustainable manner by making small slash wounds in the bark of mature trees without causing any harm to the trees. The calanolides were licensed by NCI/NIH to Medichem Research, Inc., which, as required by the NCI Letter of Collection, negotiated an agreement with the Sarawak State Government. They formed a joint venture company, Sarawak Medichem Pharmaceuticals, Inc., but the lead role in the development is now being undertaken by Craun Research Sendirian Berhad, a company incorporated in Sarawak. (+)-Calanolide A, synthesized by Medichem chemists, has shown an acceptable level of safety and a favorable pharmacokinetic profile in healthy, HIV-negative individuals, and is currently in further clinical trials, while (-)-Calanolide B is in preclinical development. The early development of the calanolides has been reviewed as a "Benefit-Sharing Case Study" for the Executive Secretary of the Convention on Biological Diversity (ten Kate and Wells, [http://www.cbd.int/abs/cs.shtml](http://www.cbd.int/abs/cs.shtml); case study number 4).

**Research Collaboration and Plant Surveys in Belize**

With the renewal of the NCI natural products program in 1986, a contract for the collection of plants in Central and South America was awarded to The New York Botanical Garden (NYBG). Shortly after the award, Dr. Michael Balick of NYBG initiated a collaboration with the Ix Chel Tropical Research Foundation under the direction of Dr. Rosita Arvigo and Dr. Gregory Shropshire, and working in close collaboration with twelve traditional healers affiliated
with the Traditional Healers Foundation of Belize. During the period from 1987 to 1996, Dr. Balick and his collaborators provided a total of 895 plants identified to genus level from Belize to the NCI. For the purpose of this chapter, we have considered samples of different parts of the same physical plant to be counted as one item. This was done not to minimize in any way the variety and number of samples provided but to simplify the discussion of the results with respect to taxonomy and activity, as in some cases, similar activities would be found in multiple plant parts whereas in others, it would only be demonstrated in one part. Thus for the purpose of all the analyses, if any part of a plant has an activity, the activity count is unity for that particular plant, irrespective of the actual number of extracts/parts.

**Taxonomic Diversity and Numbers Tested**

The 895 identified physical plants comprised 113 families, 387 genera and 577 species. Not all of the tested samples were identified to species level, but of those materials with confirmed activities, all were taken to species level. Due to the timing of extractions and taxonomy, only a subset of these materials were ever tested for their anti-HIV activity (176 plants; equivalent to 755 extracts) before the screen ceased in late 1996, and of these 176, 170 were also tested in the anti-tumor screen. In addition to those 170, another 570 giving a total of 740 (or ~83% of those identified) were tested in the antitumor screens, leaving 155 identified and a few unidentified plants still to be extracted and tested. The antitumor tests are ongoing.

**Anti-HIV Results**

This screen, though sensitive to any mechanism of action exhibited by a potential lead, had some known potential intrinsic flaws, which were common to any such assay. These were as
follows: a) If the viral particles could not be released due to a physical constraint (e.g., a carbohydrate “coating” of the infected cells), or b) were blocked from binding to an uninfected cell (again a carbohydrate or tannin interference), then the primary assay would give a positive response. That this could occur was recognized early on and a “dereplication protocol” to remove carbohydrates and tannins was devised and implemented (Cardellina II et al., 1993).

What this effectively did was to reduce the large numbers of extracts exhibiting initial "activity" (which could approach 50% for aqueous extracts), to a very small number. Following these steps and further investigations, only one plant (*Psychotria acuminata* Benth.) actually produced agents with confirmed activity, belonging to a class of compounds known as the Gutifferones (Gustafson et al., 1992). These materials, however, did not possess enough intrinsic activity to be considered as viable candidates for development as anti-HIV drugs.

These final results from the anti-HIV studies on plants collected in Belize show the danger of using preliminary “activity” data for purposes of ethnobotanical comparisons with random collections, as it was from an initial subset of this dataset that Balick surmised that ethnobotanical collections gave a significantly higher level of activity versus random collections (Balick, 1990). Once the dereplications were performed, only one sample (or two extracts from 755) actually showed definable activity. Based on other collection datasets, this level of activity is equivalent to that which would be expected to result from a random collection and survey.

**Antitumor Results**

As mentioned earlier, there is a long history of discovery of anti-tumor agents from plants, and at the initial pass, the Belize collections have had a fair level of activity. To reach this stage, materials have to pass two fairly high hurdles; an initial assay at a nominal 100µg per ml
versus 60 human tumor cell lines (covering nine cancer types), followed by a five dose assay (four log dilutions downwards from 100µg per ml) against the same lines, followed by an evaluation as to level of interest. From historical data with this screen, less than 10% of the extracts show sufficient "activity" at the one-dose phase, and about 30-50% of these are then considered active in the five-dose assay, for an overall 3-5% level of activity.

Of the 740 plants tested to date (equivalent to over 3000 extracts) from the total collection, 40 plants (61 extracts) from 27 diverse families (Table 6.1) are currently in the NCI’s Active Repository, and are either under investigation or are available for study by qualified U.S. collaborators. Extracts are only made available subject to terms of a very strict Materials Transfer Agreement (MTA; Appendix 2) which requires that any material that advances to preclinical and clinical development include the country of origin (in this particular case, Belize) in the development process (see http://dtp.nci.nih.gov/branches/npb/agreements.html; MTA, clause 9). In addition to these samples, two other plants produced either known agents, or closely related materials, which were not further investigated as leads. These agents were Cucurbitacin B isolated from a *Gonzalaguria* sp. (Fuller *et al.*, 1994) and 4'-dimethyl-deoxypodophyllotoxin from *Hyptis verticillata* Jacq. (NCI unpublished results). Four other plants were dereplicated out by using their activity profiles and the COMPARE algorithm (for details of COMPARE see Paull *et al.*, 1989). Thus, extracts of *Thevetia ahouai* (L.) A. DC. collected in both Belize and Guatemala yielded cardenolides (e.g., neriifolin) as the active agents on bioassay-guided fractionation (Descosterd *et al.*, 1994). While this class of compounds shows potent cytotoxicity, their cardiotoxicity prevents their further consideration as potential anticancer drugs.
Those extracts which do not exhibit significant activity in the cancer cell line screen are made available to qualified research organizations through the Open Repository Program for studies related to the discovery of novel agents for the treatment of all human diseases. As with extracts from the Active Repository, these extracts are only made available subject to the terms of the MTA (Appendix 2).

Table 6.1

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<th>Plant Families from Belize with (Confirmed but Unidentified) Anti-tumor Activities</th>
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<td>Flacourtiaceae</td>
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<td>Loranthaceae</td>
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<td>Melastomataceae</td>
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<td>Moraceae</td>
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<td>Myrtaceae</td>
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Reflections on the HIV and Anti-tumor Assay Results

A few comments should be made as to the assay results that we have just reported. The proportion of antitumor-active plants (after two assays and an assessment) to the total collection so far assayed is ca. 5.4% (40/740), falling in the upper range of what has been found historically through analysis of the current NCI collections. The families that demonstrated these activities are shown in Table 6.1 and cover 27 of 113 identified families from the collection.

It must be noted that if one chose to test against a single cell line (either animal or human), as has been done in the past against the murine P388 or L1210 cell lines using one concentration (usually 20µg per ml), any plant extract that demonstrated 50% inhibition of growth at this or a lower concentration would be considered “active,” and the “putative hit rate” would be much higher, though the final number of confirmed novel structures would be very low.

The analyses that we have done are quite conservative, and as the chemical structures emerge, the percentages of “putatively novel leads” will decrease. It must be emphasized that these compounds/extracts are at the very beginning of the discovery/development process and
that the odds of any one of these actually becoming a commercial drug are significantly less than 1 in 1000. In fact, when pure compounds, not extracts are entered into clinical trials, there is a significant attrition rate at the preclinical stage, where only 1 in 10 pure compounds enter clinical trials for any disease. In cancer, for every 10 that enter clinical trials, only 1 will become a commercialized drug.

The results from the anti-HIV screen, namely the isolation of one known class of compounds, the Guttiferones, with no other activities surviving dereplication, underscores the difficulties of relating ethnobotanical and/or ethnomedical information to the concepts of disease in entirely different cultures. Since the diseases that these materials were tested in (HIV and cancer) are not generally diagnosable and treated by traditional healers, collections made solely on medicinal grounds must be compared for efficacy versus random collections in relevant disease models, e.g. in a condition that is actually treated by local ethnomedicine practitioners.

What can be gained from ethnobotanical/ethnomedical collections is the knowledge that these plants have biological activity in humans and thus warrant priority in assays, though in these days of high-throughput screens where the testing of 50,000 compounds/extract per day is now feasible, such priorities may be ephemeral in practice. Only if screening for diseases that are recognizable in both cultures (superficial mycoses, for example) might such collections show a significant acceleration in the discovery/development process. Such was not the case with these collections and these assays.

DTP World Wide Web Homepage
The NCI Developmental Therapeutics Program (DTP) offers access to a considerable body of data and background information through its World Wide Web Homepage:


Publicly available data include results from the human tumor cell line screen and AIDS antiviral drug screen, the expression of molecular targets in cell lines, and 2D and 3D structural information. Background information is available on the drug screen and the behavior of “standard agents,” NCI investigational drugs, analysis of screening data by COMPARE, the AIDS antiviral drug screen, and the 3D database. It must be noted that data and information is only available on so-called “open compounds” which are not subject to the terms of confidential submission.

In providing screening data on extracts, the extracts are identified by code numbers only; details of the origin of the extracts, such as source organism taxonomy and location of collection, may only be obtained by individuals or organizations prepared to sign agreements binding them to terms of confidentiality and requirements regarding collaboration with, and compensation of, source countries. Such requirements are in line with the NCI commitments to the source countries through its Letter of Collection and the Material Transfer Agreement.
References


National Cancer Institute. Unpublished Results.


