Natural Product Drug Discovery and Development: the United States National Cancer Institute Role

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Over the ages, humans have relied on nature for their basic needs for the production of foodstuffs, shelters, clothing, means of transportation, fertilizers, flavors and fragrances, and not least, medicines. Plants have formed the basis of sophisticated traditional medicine systems that have been in existence for thousands of years in countries, such as China (1) and India (2). These plant-based systems continue to play an essential role in health care, and it has been estimated by the World Health Organization that approximately 80% of the world's inhabitants rely mainly on traditional medicines for their primary health care (3). Plant products also play an important role in the health care systems of the remaining 20% of the population mainly residing in developed countries. Analysis of data on prescriptions dispensed from community pharmacies in the United States from 1959 to 1980 indicates that about 25% contained plant extracts or active principles derived from higher plants, and at least 119 chemical substances, derived from 90 plant species, can be considered as important drugs currently in use in one or more countries (3). Of these 119 drugs, 74 % were discovered as a result of chemical studies directed at the isolation of the active substances from plants used in traditional medicine. Well-known examples of plant-derived medicinal agents include the antimalarial drug quinine, obtained from the bark of *Cinchona officinalis*, the analgesics, codeine and morphine from *Papaver somniferum*, the antihypertensive reserpine from *Rauwolfia serpentina*, and the cardiac glycoside, digoxin from *Digitalis purpurea* (4).

While marine organisms do not have a history of use in traditional medicine, the ancient Phoenicians employed a chemical secretion from marine molluscs to produce purple dyes for woolen cloth, and seaweeds have long been used to fertilize the soil. The world's oceans, covering more than 70% of the earth's surface, represent an enormous resource for the discovery of potential chemotherapeutic agents. All but two of the 28 major animal phyla are represented in aquatic environments, with eight being exclusively aquatic, mainly marine (5).

The discovery of penicillin from the filamentous fungus, *Penicillium notatum*, and the broad therapeutic use of this agent in the 1940s, ushered in a new era in medicine and the "Golden Age" of antibiotics, and promoted the intensive investigation of nature as a source of novel bioactive agents. Microorganisms are a prolific source of structurally-diverse bioactive metabolites and have yielded some of the most important products of the drug industry, including the penicillins, aminoglycosides, tetracyclines, cephalosporins, and other classes of antibiotics that have revolutionized modern medicine.

This interest in nature as a source of potential chemotherapeutic agents continues, and an analysis of the number and sources of anticancer and anti-infective agents, reported mainly in the Annual Reports of Medicinal Chemistry from 1984 to 1995 covering the years 1983 to 1994, indicates that over 60% of the approved drugs developed in these disease areas are of natural origin (6).

Anticancer Agents Derived From Natural Sources : the NCI Role. 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

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component of the Division of Cancer Treatment and Diagnosis (DCTD). 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. It has played a major role in the discovery and development of many of the available commercial and investigational anticancer agents. During this period, more than 400,000 chemicals, both synthetic and natural, have been screened for antitumor activity.

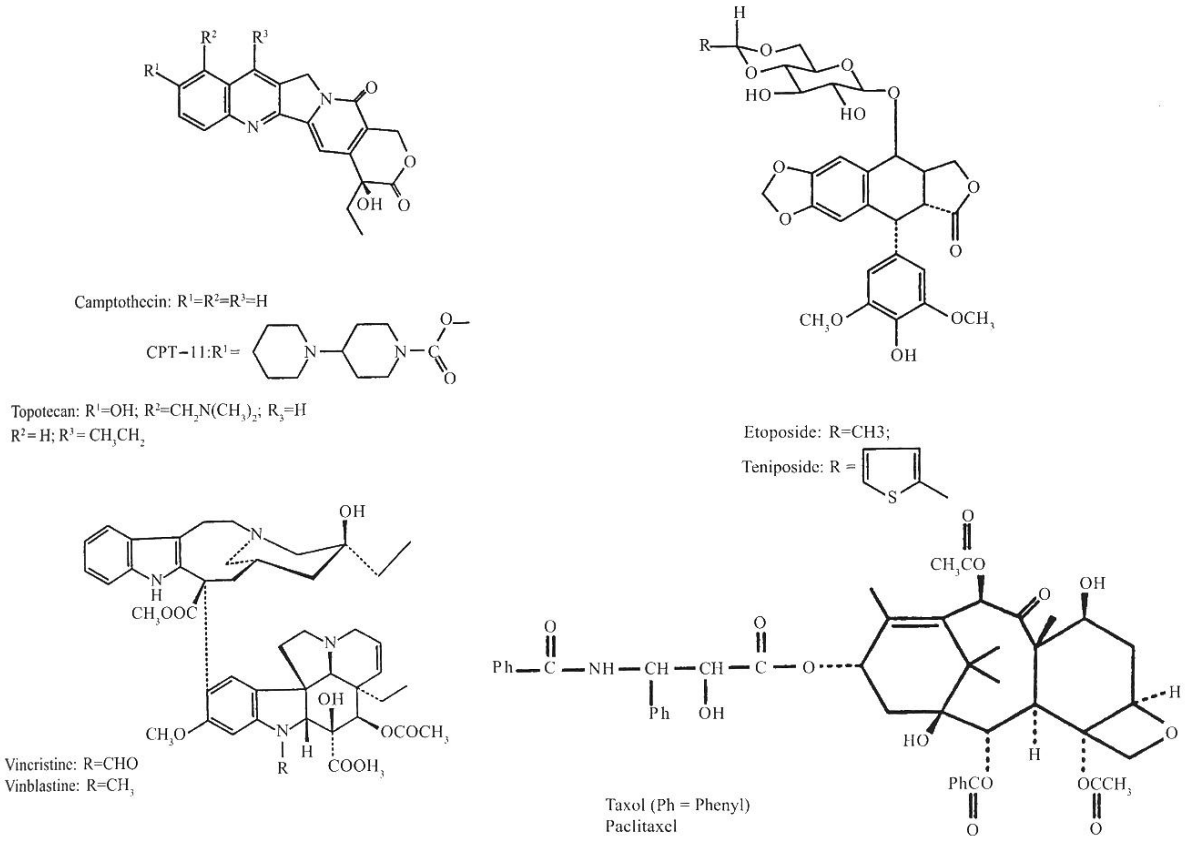
Initially, most of the materials screened were pure compounds of synthetic origin, but the program also recognized that natural products were an excellent source of complex chemicals with a wide variety of biological activities. During the early years of the CCNSC, the screening of natural products was concerned mainly with the testing of microbial fermentation products, and, prior to 1960, only about 1,500 plant extracts were screened for antitumor activity. Plants have a long history of use in the treatment of cancer (7), 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 (8). 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, 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, however, provided convincing evidence that plants could be sources of a variety of novel potential cancer chemotherapeutic agents (Figure 1) (8); epipodophyllotoxin, isolated as the active antitumor agent from various species of *Podophyllum,* was semisynthetically converted into the clinically active agents, etoposide and teniposide. Thus the 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 (9). Those agents showing significant activity in the secondary panel were assigned priorities for preclinical and clinical development.

Of the 92 anticancer drugs commercially available prior to 1983 in the United States and approved worldwide between 1983 and 1994, approximately 62% can be related to natural origin (6). 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. Two plant-derived agents, paclitaxel (Taxol®) and camptothecin, were discovered through the NCI program, and, while camptothecin failed as a clinical candidate in the 1970s, its derivatives, topotecan, 9-amino camptothecin and irenotecan (CPT-11), are currently showing clinical efficacy against a variety of cancer disease types (Figure 1) (10). Other plant-derived drugs in clinical trials are homoharringtonine isolated from the small Chinese evergreen tree, *Cephalotaxus harringtonia* var. *Drupacea* (Sieb. & Zucc.) *Koidzumi*, and 4-ipomeanol, a pneumotoxic furan derivative produced by sweet potatoes (*Ipomoea batatas*) infected with the fungus, *Fusarium solani*. Homoharringtonine has shown activity against various leukemias, while ipomeanol is in early clinical trials for treatment of patients with lung cancer (11). A number of plant-derived agents were entered into clinical trials by the NCI, but the trials were terminated due to lack of efficacy or unacceptable toxicity. Amongst these agents were acronycine, bruceantin, maytansine and thalicarpine, all of which could serve as cytotoxins for linking to monoclonal antibodies or other "carrier" molecules targeted to specific tumors.

Many of the commercial drugs of microbial origin, such as actinomycin D, doxorubicin (adriamycin) and mitomycin C, were discovered by research groups associated with the pharmaceutical industry, and this trend continues, generally in close collaboration with the NCI in the developmental phases. Much of the drug discovery effort in the marine area, however, has been supported by the NCI through contract or grant mechanisms. While no marine-organism-derived agent has yet been approved for commercial development, several agents, including bryostatin 1 and dolastatin 10, are in clinical trials (12); bryostatin 1 is showing some promising activity in trials against melanoma (Figure 2) (13).

Most of the drugs currently available for cancer therapy are effective predominantly against rapidly proliferating tumors, such as leukemias and lymphomas, but, with some

Figure 1. Commercial Plant-Derived Anticancer Drugs



notable exceptions such as paclitaxel, show little useful activity against the slow-growing adult solid tumors, such as lung, colon, prostatic, pancreatic, and brain tumors. In the early 1980s, the NCI program was discontinued because it was perceived that few novel active leads were being isolated from natural sources. Of particular concern was the failure to yield agents possessing activity against the 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 appeared to be detecting only previously identified active compounds or chemical structure types having little or no activity against solid tumors.In retrospect, these results might be attributed to the use of a single disease-specific model as the primary screen that filtered out those agents with potential specificity against tumors other than mouse leukemia or closely related human diseases.

In an attempt to overcome this deficiency, NCI has developed an alternative, disease-oriented, preclinical anticancer drug discovery strategy aimed at the discovery of new agents for disease-specific clinical trials in relevant cancer patient populations (14).

Current Status of the NCI Natural Products Drug Discovery Program. During 1985-1990 the NCI developed a new *in vitro* primary screen based upon a diverse panel of human tumor cell lines (14). The screen currently comprises sixty cell lines derived from nine cancer types, and organized into subpanels representing leukemia, lung, colon, central nervous system, melanoma, ovarian, renal, prostate and breast. As of late 1998, preliminary prescreen comprising three cell lines was be introduced, and all materials are tested in the prescreen. Those materials showing significant activity in one or more of the three lines are advanced to the sixty cell line screen for further evaluation.

With the development of the new *in vitro* screening strategy, the NCI once again turned to nature as a potential source of novel anticancer agents, and a new natural products acquisition program was implemented in 1986. Contracts for the cultivation and extraction of fungi and

cyanobacteria, and for the collection of marine invertebrates and terrestrial plants, were initiated in 1986, and with the exception of fungi and cyanobacteria, these programs continue to operate. Marine organism collections originally focused in the Caribbean and Australasia, but have now expanded to the Central and Southern Pacific and to the Indian Ocean (off East and Southern Africa) through a contract with the Coral Reef Research Foundation, which is now based in Palau in Micronesia. Terrestrial plant collections have been carried out in over 25 countries in tropical and subtropical regions worldwide through contracts with the Missouri Botanical Garden (Africa and Madagascar), the New York Botanical Garden (Central and South America), and the University of Illinois at Chicago (Southeast Asia), and have been expanded to the continental United States through a contract with the Morton Arboretum.

In carrying out these collections, the NCI contractors work closely with qualified organizations in each of the source countries. Botanists and marine biologists from source country organizations collaborate in field collection activities and taxonomic identifications, and their knowledge of local species and conditions is 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's Natural Products Repository (NPR) in Frederick, Maryland. The collaboration between the source country organizations and the NCI collection contractors, in turn, provides 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 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 (15). Contract collections of plants are now being de-emphasized in favor of establishing direct collaborations with qualified organizations in the source countries; these developments are discussed in Section 5.3.

Dried plant samples (0.3-1 kg dry weight) and frozen marine organism samples (~ 1 kg wet weight) are shipped to the NPR in Frederick where they are stored at -20°C prior to extraction with a 1:1 mixture of methanol: dichloromethane 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. After testing in the *in vitro* human cancer cell line screen, 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 in several *in vivo* systems. Those agents exhibiting significant *in vivo* activity are advanced into preclinical and clinical development. Of the 42 anticancer agents currently in active preclinical or Phase I development (excluding biologics), 23 are either natural products or derived from natural products, with the source organisms being 14 microbial, 2 marine, 3 plant and 3 animal in origin, together with one pyrimidine base (6).

As part of the response of the National Institutes of Health (NIH) to the AIDS epidemic, DTP developed a screening program for the large-scale testing of synthetic and natural materials for anti-HIV activity (16). The screen measured the effect of materials on the growth of human lymphoblastoid cells in the presence or absence of the human immunodeficiency virus (HIV-1) (17), and from 1988 to 1996, extracts were tested in this screen. As of late 1996, the screening of extracts was discontinued, and alternative assays involving the use of target enzymes are now being used.

Preclinical development. Those agents showing significant *in vivo* activity are presented to the NCI Drug Development Group (DDG) and, if approved by the DDG, the agent is entered into preclinical and clinical development.

Preclinical development includes:

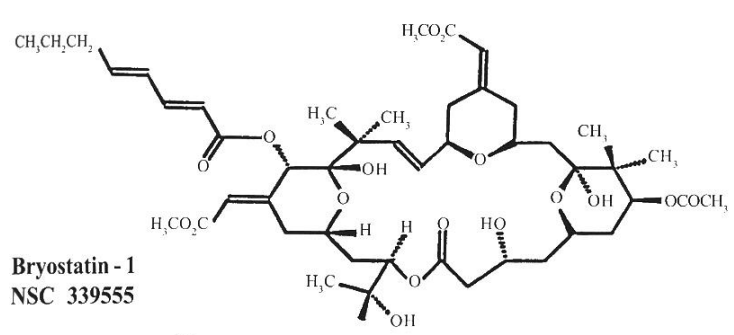
- The procurement of an adequate supply of any natural product drug to permit preclinical and clinical development.
- Formulation studies are performed to develop a suitable vehicle to solubilize the drug to enable administration to patients, generally by intravenous injection or infusion in the case of cancer. The low solubility of many natural products in water poses considerable problems, but these can be overcome by use of co-solvents or emulsifying agents (surfactants) such as Cremophore EL (polyoxyethylated castor oil).
- Pharmacological evaluation involves the determination of 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.

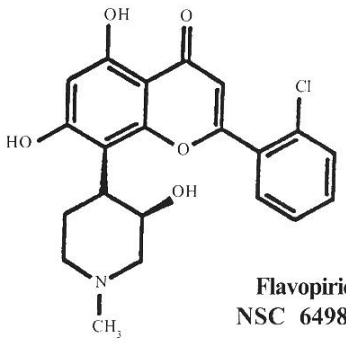
- In the final preclinical step, toxicological studies are performed to determine the type and degree of major toxicities in rodent and dog models. These studies help to establish the safe starting doses for administration to human patients in clinical trials.

Clinical development. Phase I studies are conducted to determine the maximum tolerated dose (MTD) of a drug in humans, and observe the sites and reversibility 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

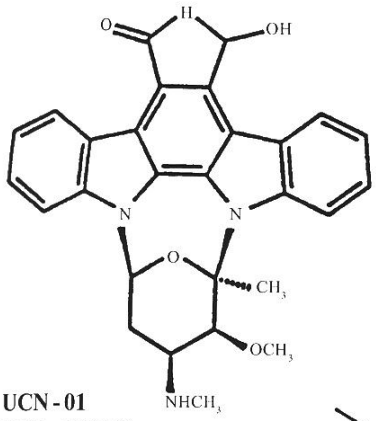
Figure 2. Some Natural Product - Derived Anticancer Compounds Under Development



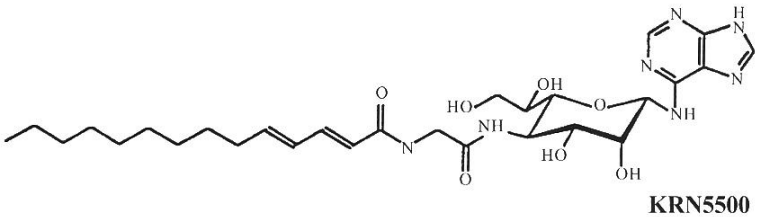
Bryostatin - 1
NSC 339555



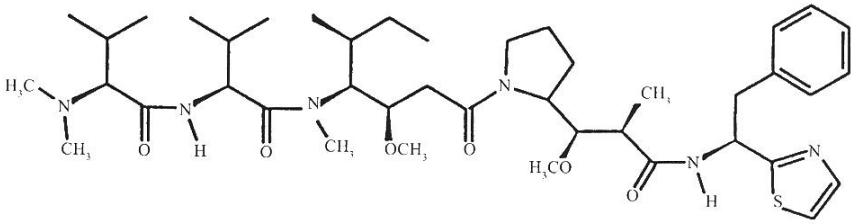
Flavopiridol
NSC 649890



UCN - 01
NSC 638850



KRN5500
NSC 650426



Dolastatin 10
NSC 376128

that of the individual drugs used alone.

Natural Product Drug Development : The Supply Issue. The critical first step in the development of any natural product drug is the procurement of an adequate supply to meet the requirements for preclinical and clinical investigation. While total synthesis may be considered as a potential route for bulk production of the active agent, it is worth noting that the structures of most bioactive natural products are extremely complex, and bench-scale syntheses often are not readily adapted to large-scale economic production. Isolation from the natural source, therefore, often provides the most economically viable method of production. It should be noted that, of the established plant-derived commercial anticancer drugs, vinblastine and vincristine are still produced by isolation from *Catharanthus roseus* grown in various regions worldwide, while etoposide and teniposide are semisynthetically produced from natural precursors isolated from *Podophyllum emodii* harvested in India and Pakistan (Figure 1). The problems associated with the large-scale production of paclitaxel have also been resolved through semisynthesis from natural precursors, such as baccatin III and 10-desacetylbaccatin III, isolated from the needles of various *Taxus* species (18).

The initial raw material collection sample (0.3-1.0 kg) will generally yield enough extract (10-40 g) to permit isolation of the pure, active constituent in sufficient milligram quantity for complete structural elucidation. Subsequent secondary testing and preclinical development, however, might require gram or even kilogram quantities, depending on the degree of activity and toxicity of the active agent.

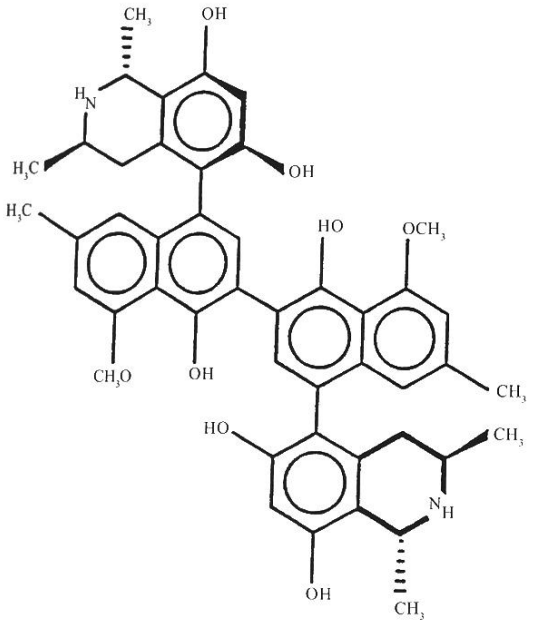
In order to obtain sufficient quantities of an active agent for early preclinical development, recollections of 5-50 kg of the raw material, preferably from the original collection location, might be necessary. Should the preclinical studies justify development of the agent towards clinical trials, considerably larger amounts of material would be required. The performance of large recollections necessitates surveys of the distribution and abundance of source organism as well as determination of the variation of drug content in the various parts in the case of plants, and the fluctuation of content with the time and season of harvesting. In addition, the potential for mass cultivation or aquaculture of the source organism would need to be assessed. If problems are encountered due to scarcity of the wild source organism or inability to adapt it to cultivation, a search for alternative sources would be necessary. Other species of the same genus, or closely related genera, can be analyzed for drug content, and techniques, such as tissue culture, can be investigated.

The discovery and development of paclitaxel (Taxol®). Probably the most significant drug discovered and developed through the NCI natural products program is the plant-derived agent, paclitaxel . Paclitaxel was isolated through an NCI contract by Drs. Monroe Wall and Mansukh Wani of Reserach Triangle Institute from the bark of the Pacific Yew tree (*Taxus brevifolia)* in 1969 from samples collected by the USDA as part of the early exploratory plant screening program. Like many other potential anticancer agents at that time, paclitaxel only showed moderate activity against the then current mouse leukemia models, and was not considered of particular interest. It was only the observation of its activity in new test systems (the B16 melanoma and several human tumor xenografts) developed in the mid-to late 1970s that revived interest. This interest was further heightened by the discovery of its unique mechanism of action by Dr. Susan Horwitz of Albert Einstein School of Medicine; paclitaxel polymerizes and stabilizes microtubules, thereby inhibiting mitosis and cell division.

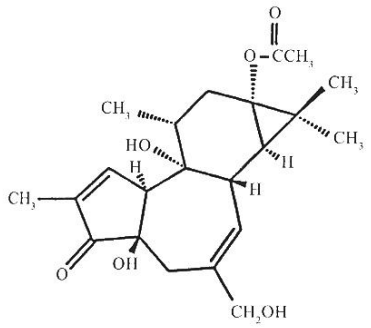
These observations promoted the development of paclitaxel which advanced through preclinical studies (e.g. animal toxicology) to initiation of Phase I clinical trials in 1983. The early trials were fraught with serious problems of toxicity, particularly allergic reactions including anaphylaxis, which brought it very close to being dropped from clinical studies for safety reasons. The toxicity was traced back to the very poor solubility of taxol in aqueous systems which required the use of high concentrations of the emulsifying agent, Cremophore EL (a castor oil derivative), in the preparation of a suitable vehicle for parenteral administration of the drug; Cremophore EL is known to illicit hypersensitivity reactions. These problems were alleviated by the use of longer infusion times (e.g. 24 hours every 14-21 days) and premedication with anti-allergy drugs.

Due to the slow progress of paclitaxel through Phase I clinical trials and doubts about its clinical efficacy, only sufficient drug for a moderate number of trials was isolated in bulk (several kilograms) from the Pacific Yew bark. This later became a problem when important activity was found in Phase II trials in ovarian cancer in 1987 and interest in the drug greatly intensified. The observation of approximately 30% response rates in trials with patients having refractory ovarian cancer resulted in a tremendous demand for the drug. The yield of paclitaxel isolated was about 1 gram per 30lbs of bark, and the average Pacific Yew tree (about 100 years in age) yielded about 20lbs of bark (equivalent to 1.5 trees per gram). Given that about 12,000 women were dieing annually in the U. S. from advanced ovarian cancer, and the usual treatment required about 2 grams of paclitaxel per patient, 24,000 grams (24Kg) were required, amounting to the destruction of 36,000 trees. Meanwhile, significant activity was also observed in the

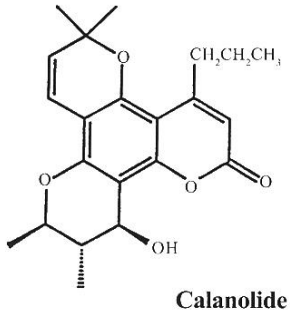
Figure 3. Some Natural Product-Derived Anti-AIDS Leads Discovered by the NCI



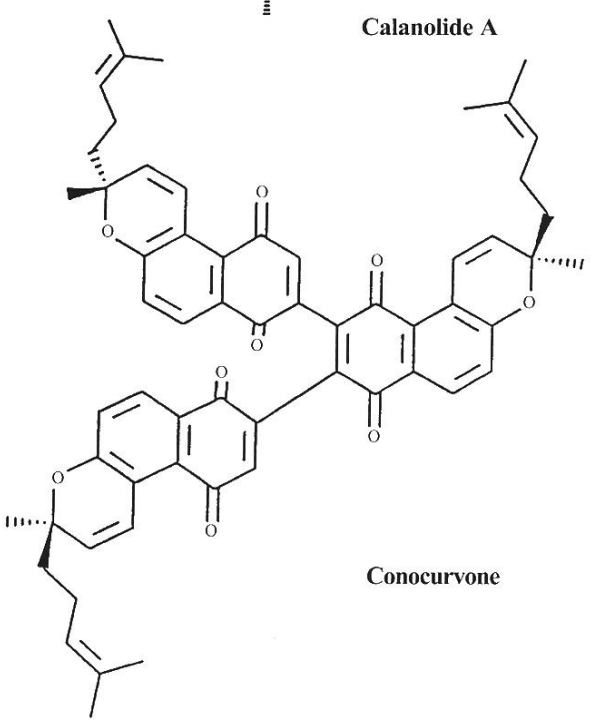
Michellamine B



Prostratin



Calanolide A



Conocurvone

treatment of patients with metastatic breast cancer (40,000 deaths per year), and responses were also being observed in patients with other forms of advanced malignancy, including lung cancer, malignant melanoma, and lymphomas.

A detailed analysis of the paclitaxel supply crisis and its eventual solution has been published (16), so only a brief review is presented here. The initial source of paclitaxel was the bark of the Pacific Yew, *Taxus brevifolia*, an understorey tree growing in the forests of the Pacific northwest from northern California into British Columbia. The taxol supply needs for preclinical and early clinical studies were easily met by bark collections in Oregon between 1976 and 1985, ranging in size from 2,000 pounds to 15,000 pounds. Later observations of responses in the treatment of patients with a variety of solid tumors, including malignant melanoma and ovarian cancer, led to an escalation in demand for drug, resulting in several 60,000 pound-collections between 1987 and 1989. These collections raised concerns about their impact on the continued existence of the tree, but inventories conducted by the USDA Forest Service and Bureau of Land Management and funded by Bristol-Myers Squibb (BMS) determined that the tree was abundant (estimates of >100 million trees) on government land. Over 1.6 million pounds of bark were harvested under strictly controlled conditions in each of the years 1991 and 1992 by Hauser Northwest, a subsidiary of Hauser Chemical Research (HCR) under

contract to BMS. These collections resulted in the production of hundreds of kilograms of paclitaxel by HCR.

Both NCI and BMS realized that alternative sources of paclitaxel would need to be developed to permit its eventual marketing as a clinical drug, and NCI organized workshops in 1990 and 1992 to promote research into various aspects of paclitaxel. Analytical surveys of the needles of a number of *Taxus* species collected from several countries, including Canada, Georgia, Mexico, Russia, Ukraine and the United States, were performed, and the content of paclitaxel and key baccatin precursors in various *Taxus* cultivars was determined. Though the paclitaxel content of the needles was generally lower than that of the bark, needles of several species and cultivars were found to be relatively good sources of baccatin precursors. The pioneering studies, by the French research team of Greene, Poitier and coworkers, of the semisynthetic conversion of 10-desacetylbaccatin III, isolated from the needles of *T. baccata*, to paclitaxel, and the subsequent development of more efficient conversion processes, has led to the large-scale production of paclitaxel and related compounds, such as taxotere (19), from renewable needle resources, and the solution of the supply problem.

Significant advances have also been made in the production of paclitaxel through plant tissue culture using technology developed by the company Phyton Catalytic, working with BMS.

Development of the potential anti-HIV agent, michellamine B. Michellamine B (Fig. 3) was isolated as the main *in vitro* active anti-HIV agent from the leaves of the liana, *Ancistrocladus korupensis*, collected in the Korup region of southwest Cameroon. Initially the plant was tentatively identified as *A. abbreviatus*, but collections of this and all other known *Ancistrocladus* species failed to yield any michellamines or show any anti-HIV activity. Subsequent detailed taxonomic investigation of the source plant compared to authentic specimens of *A. abbreviatus* revealed subtle but distinctive morphological differences, and the species was determined to be new to science, and officially named *Ancistrocladus korupensis* (20). Michellamine B shows *in vitro* activity against a broad range of strains of both HIV-1 and HIV-2, including several resistant strains of HIV-1 (21). The species appears to be mainly distributed within the Korup National Park, and vine densities are of the order of one large vine per hectare. Fallen leaves collected from the forest floor do contain michellamine B, and collections of these leaves provided sufficient biomass for the isolation of enough drug for completion of preclinical development. It was clear, however, that extensive collections of fresh leaves could pose a possible threat to the wild source. Thus far, no other *Ancistrocladus* species has been found to contain michellamine B, and investigation of the feasibility of cultivation of the plant as a reliable biomass source was initiated in 1993 through a contract with the Center for New Crops and Plant Products of Purdue University working in close collaboration with the University of Yaounde 1, the World Wide Fund for Nature Korup Project, Missouri Botanical Garden, Oregon State University and the NCI contractor, SAIC. An extensive botanical survey was undertaken, and the range and distribution of the species were mapped out. Dried leaf samples from representative vines were shipped to NCI for analysis of michellamine B content. Plants indicating high concentrations were re-sampled for confirmatory analysis, and those showing repeated high concentrations were targeted for cloning via vegetative propagation. A medicinal plant nursery was established to hold and maintain the *A. korupensis* collection at the Korup Park Headquarters in Mundemba. In keeping with the NCI policies of collaboration with source countries, all the cultivation studies were performed in Cameroon, and involved the local population, particularly those in the regions adjacent to the Korup National Park.

Based on the observed activity, the NCI committed michellamine B to INDA-directed preclinical development. Unlike many natural products, formulation presented no problem since the drug is readily water-soluble as its diacetate salt. Continuous infusion studies in dogs indicated that *in vivo* effective anti-HIV concentrations could only be achieved close to toxic dose levels. Thus, despite these observations and the *in vitro* activity against an impressive range of HIV-1 and HIV-2 strains, there were some serious disadvantages which precluded advancement of michellamine B to clinical trials. The difference between the toxic dose level and the anticipated level required for effective antiviral activity was small, indicative of a very narrow therapeutic index. Further toxicology studies in primates confirmed the very narrow therapeutic index, and indicated potential neurological toxicity. Based on these observation, NCI decided to discontinue further studies aimed at clinical development.

Despite this decision, it is possible that the pharmacological and toxicological profiles can be improved through analogue synthesis. Such studies could require substantial quantities of the natural product, or the successful synthetic studies of Bringmann and his group could provide a satisfactory solution (22). The isolation of the novel antimalarial compounds, the korupensamines, from *A. korupensis*, provides another class of potential medicinal agents from this plant (23). The korupensamines, which are equivalent to the "monomeric" units of the michellamines, are essentially inactive against HIV, whereas the michellamines exhibit only very weak antimalarial

activity.

Collaboration In Drug Discovery and Development : the NCI Role. As noted above, much of the NCI drug discovery and development effort has been, and continues to be, carried out through collaborations with research organizations and the pharmaceutical industry worldwide.

Many of the naturally derived anticancer agents were developed through such collaborative efforts. Thus, the discovery and preclinical development of etoposide and teniposide, semisynthetic derivatives of the natural product epipodophyllotoxin, were performed by Sandoz investigators, and the NCI played a substantial role in the clinical development. Though paclitaxel (Taxol®) was discovered by Wall and Wani with NCI contract support, the key to solving the supply problem was the semisynthetic conversion of baccatin III derivatives to paclitaxel (and taxane analogs) pioneered by the French group led by Poitier, followed by the development of alternative conversion methods by the Holton group, supported by the NCI, and Bristol-Myers Squibb (18). The semisynthetic analog, taxotere (docetaxel), produced through a collaborative agreement between the Centre National de la Recherche Scientifique (CNRS) and Rhone-Poulenc Rorer, after undergoing extensive clinical evaluation in Europe and North America under auspices of organizations, such as the European Organization for Research and Treatment of Cancer (EORTC), and the Canadian and U.S. National Cancer Institutes (19) is now in clinical use in Europe and North America. Indeed, there is close collaboration between the EORTC, the United Kingdom Cancer Research Campaign (CRC) and the NCI in the preclinical and clinical development of many anticancer agents, including agents such as bryostatin 1, dolastatin 10, aphidicolin glycinate, rhizoxin, pancratistatin and phyllanthoside.

Drugs, such as bleomycin, aclacinomycin and deoxyspergualin, were discovered by the Umezawa group at the Institute of Microbial Chemistry in Japan and developed in collaboration with the NCI; a number of the agents currently in advanced preclinical development at the NCI, such as UCN-01, and quinocarmycin and spicamycin analogs, are the result of collaborations between Japanese companies, such as Kyowa Hakko Kogyo, Fujisawa Pharmaceutical Co. Ltd, and Kirin Brewery Ltd, and the NCI (12).

The DTP of the NCI thus complements the efforts of the pharmaceutical industry and other research organizations through undertaking positive leads, which industry might consider too uncertain to sponsor, and conducting the "high risk" research necessary to determine their potential utility. In promoting drug discovery and development, the DTP/NCI has formulated various mechanisms for establishing collaborations with research groups worldwide.

Screening agreement between compound providers and the NCI DCTD. In the case of organizations wishing to have pure compounds tested in the NCI drug screening program, such as pharmaceutical and chemical companies or university research groups, the DTP/NCI has formulated a screening agreement which includes terms stipulating confidentiality, patent rights, routine and non-proprietary screening and testing versus non-routine and proprietary screening and testing, and levels of collaboration in the drug development process. Individual scientists and research organizations wishing to submit pure compounds for testing generally consider entering into this agreement with the NCI DCTD. Details are given on the DTP website.

Should a compound show promising anticancer activity in the routine screening operations, the NCI will propose the establishment of a more formal collaboration, such as a Cooperative Research and Development Agreement (CRADA) or a Clinical Trial Agreement (CTA).

Collaboration in preclinical development: rapid access to intervention development(RAID). RAID is a new program designed to facilitate translation to the clinic of novel, scientifically meritorious therapeutic interventions originating in the academic community. The RAID process makes available to the academic research community, on a competitive basis, NCI resources for preclinical development of drugs. The process functions as a collaboration between the NCI and the originating laboratory, and tasks may be apportioned to either the NCI or the originating laboratory, depending on the facilities and expertise available in the latter. While the RAID process is similar to the Decision Network Process, the products of the RAID program are returned directly to the originating laboratory for proof-of-principle clinical trials. It is assumed that most of the products in the RAID program will be studied clinically under investigator-held INDs (Investigational New Drug approvals granted by the FDA) within the originating (or a collaborating) institution. NCI may consider assuming responsibility for clinical trials sponsorship if unanticipated circumstances develop precluding clinical development by the originating institution. The RAID process cannot be used by private industry (which can interact with NCI through the DN process), nor can it be used to develop a product already licensed to a company; however, the existence of research collaborations between the academic investigators and companies does not affect the eligibility for support from RAID for an individual product, provided the product is not licensed to a company.

Full details may be obtained from the DTP Website.

National Cooperative Drug Discovery Group (NCDDG)

Program. In the late 1970s and early 1980s, many significant discoveries were being made in such fields as biochemistry, molecular biology, embryology and carcinogenesis, that had the potential for the development of new strategies and agents for cancer treatment; most investigators, however, were working only in their own areas of expertise without the benefit of close liaison with experts in the many disciplines required to discover and develop new therapies and strategies. In response to the need to coordinate these research efforts, the NCI initiated the NCDDG Program in the early 1980s with the goal of bringing together scientists from academia, industry and government, in the form of consortia, in a focused effort aimed at the discovery of new drugs for cancer treatment (24). The inclusion of an industrial component in almost all consortia has had strong positive effects in helping to orient the academic component(s) towards drug development, and maintaining a focus on the final outcomes of drug discovery in terms of clinical trials and marketable products, as well as contributing high quality scientists and resources to the Program. Involvement of NCI Staff has enabled the NCI to contribute its considerable resources and expertise in cancer drug development, including extensive computerized databases and repositories of compounds tested over more than 35 years, primary and secondary screening systems, and all the resources necessary for preclinical development of agents meeting selection criteria of the NCI Decision Network Committee. The consortia, headed by a Principal Investigator, submit proposals based on independent ideas, rather than in response to specific topics proposed by the NCI, thereby permitting the widest scope and the greatest degree of innovative science, and encouraging diversity in the discovery of new drugs and therapeutic approaches.

The National Cooperative Natural Product Drug Discovery Group (NCNPDDG) Program is one of four such programs, the other three being directed at studies of Mechanisms of Action, Specific Diseases (e.g., lung and colon cancer), and Preclinical Model Development. Since 1989, twelve NCNPDDGs have been awarded encompassing the study of all natural sources, including plants, marine bacteria and invertebrates, microalgae, cyanophytes and dinoflagellates, and using a variety of assays, such as molecular targets, mechanism-based assays, cell lines and *in vivo* systems.

Source Country Collaboration. As discussed earlier, the collections of plants and marine organisms have been carried out in over 25 countries through contracts with qualified botanical and marine biological organizations working in close collaboration with qualified source country organizations. 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 in aiding the performance of the NCI collection programs, have led the NCI to formulate its LOC specifying policies aimed at facilitating collaboration with, and compensation of, countries participating in the NCI drug discovery program (15).

With the increased awareness of genetically-rich source countries to the great value of their natural resources and the confirmation of source country sovereign rights over these resources by the U.N. Convention of Biological Diversity, organizations involved in drug discovery and development are increasingly adopting policies of equitable collaboration and compensation in interacting with these countries (25). Particularly in the area of plant-related studies, source country scientists and governments are committed to performing more of the operations in-country, as opposed to the export of raw materials. The NCI has recognized this fact for several years, and has negotiated Memoranda of Understanding (MOU) with a number of source country organizations suitably qualified to perform in-country processing. In considering the continuation of its plant-derived drug discovery program, the NCI has de-emphasized its contract collection projects in favor of expanding closer collaboration with qualified source country scientists and organizations. In establishing these collaborations, NCI undertakes to abide by the same policies of collaboration and compensation, as specified in the LOC. A number of other organizations and companies have implemented similar policies (25).

Through this mechanism collaborations have been established with organizations in Bangladesh, Brazil, China, Costa Rica, Iceland, Korea, Mexico, New Zealand, Pakistan, Panama, Russia, South Africa, and Zimbabwe.

In 1988, an organic extract of the leaves and twigs of the tree, *Calophyllum lanigerum*, collected in Sarawak in 1987, through the NCI contract with the University of Illinois at Chicago (UIC) in collaboration with the Sarawak Forestry Department, showed significant anti-HIV activity. Bioassay-guided fractionation of the extract yielded (+)-calanolide A (Figure 3) as the main *in vitro* active agent (26). Attempted recollections in 1991 failed to locate the original tree, and collections of other specimens of the same species gave only trace amounts of calanolide A. In 1992, a detailed survey of *C. lanigerum* and related species was undertaken by UIC and botanists of the Sarawak Forestry Department. As part of the survey, latex samples of *Calophyllum teysmanii* were collected, and yielded extracts with showing significant anti-HIV activity. The active constituent was found to be (-)-calanolide B which

was isolated in yields of 20 to 30%. While (-)-calanolide B was 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. A decision was made by the NCI DNC to proceed with the preclinical development of both of the calanolides, and, in June of 1994, an agreement based on the NCI Letter of Collection was signed between the Sarawak State Government and the NCI. Under the agreement a scientist from the University of Malaysia Sarawak was invited to visit the NCI laboratories in Frederick to participate in the further study of the compounds.

The NCI obtained patents on both calanolides, and, in 1995, an exclusive license for the development of the calanolides was awarded to Medichem Research, Inc., a small pharmaceutical company based near Chicago. Medichem Research had developed a synthesis of (+)-calanolide A (27) under a Small Business Innovative Research (SBIR) grant from the NCI. The licensing agreement specified that Medichem Research negotiate an agreement with the Sarawak State Government. Medichem Research, in collaboration with the NCI, has advanced (+)-calanolide A through preclinical development and has been granted an INDA for clinical studies by the U. S. Food and Drug Administration (FDA). The Sarawak State Government and Medichem Research formed a joint venture company, Sarawak Medichem Pharmaceuticals Incorporated (SMP) in late1996, and SMP sponsored Phase I clinical studies with healthy volunteers in which it has been shown that doses exceeding the expected levels required for efficacy against the virus are well tolerated. Trials with patients infected with HIV-1 are currently in progress.

Meanwhile, by late 1995 the Sarawak State Forestry Department, UIC and the NCI had collaborated in the collection of over 50 kg of latex of *C. teysmanii*, and kilogram quantities of (-)-calanolide B have been isolated for further development towards clinical trials. The development of the calanolides is being facilitated through the signing of a Cooperative Research and Development Agreement (CRADA) between Medichem Research and the NCI in which NCI is contributing considerable research knowledge and expertise.

The development of the calanolides is an excellent example of collaboration between a source country (Sarawak, Malaysia), a company (Medichem Research, Inc.) and the NCI in the development of promising drug candidates, and illustrates the effectiveness and strong commitment of the NCI to policies promoting the rights of source countries to fair and equitable collaboration and compensation in the drug discovery and development process. The development of the calanolides has been reviewed as a "Benefit-Sharing Case Study" for the Executive Secretary of the Convention on Biological Diversity by staff of the Royal Botanic Gardens, Kew (28).

Distribution of extracts from the NCI Natural Products Repository. In carrying out the collection and extraction of thousands of plant and marine organism samples worldwide, the NCI has established a Natural Products Repository (NPR) which is a unique and valuable resource for the discovery of potential new drugs and other bioactive agents. The rapid progress made in the elucidation of mechanisms underlying human diseases has resulted in a proliferation of molecular targets available for potential drug treatment. The adaptation of these targets to high throughput screening processes has greatly expanded the potential for drug discovery. In recognition of this potential, the NCI has developed policies for the distribution of extracts from the NPR to qualified organizations for testing in screens related to all human diseases, subject to the signing of a legally-binding Material Transfer Agreement (MTA) (see DTP Homepage).

To be considered for access to the NPR, organizations are required to submit short proposals outlining the nature of their screening systems and demonstrating the capability to process active extracts and develop any isolated active agents towards clinical trials and commercial production. Approved organizations have to enter into an MTA with DCTD, with one of the key terms being the requirement for the recipient organization to negotiate suitable terms of collaboration and compensation with the source country(ies) of any novel agent isolated and entered into preclinical and clinical development.

DTP WWW Homepage. The NCI DTP offers access to a considerable body of data and background information through its Homepage: http://dtp.nci.nih.gov

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 (14), the AIDS antiviral drug screen, and the 3D database. It must be noted that data and information are 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 LOC and the MTA.

International Cooperative Biodiversity Group (ICBG) Program. A multiagency program. The ICBG Program resembles NCDDG Programs in structure, in that consortia are formed comprising academic, industrial and U.S. government organizations, but organizations from developing countries are also required components. This Program is jointly sponsored by the National Science Foundation (NSF) and components of the National Institutes of Health (NIH), including the NCI, the National Institute of Allergy and Infectious Diseases (NIAID), the National Heart, Lung and Blood Institute (NHLBI), and the National Institute of Mental Health (NIMH). The goals of the Program are research into drug discovery from natural sources, linked to the identification, inventory and conservation of biodiversity, a primary concern of the NSF, and economic development in developing countries (24). All these goals are linked to the provision of suitable training and infrastructure building.

Five awards, four involving countries in Central and South America and one involving the West African countries of Cameroon and Nigeria, were awarded in 1993 and 1994. The program was recompeted in 1998, and projects involving Madagascar, Vietnam and Laos are now also in operation. The program is administered through the NIH Fogarty International Center. A significant challenge in the development of the ICBGs was the establishment of principles related to intellectual property rights and the protection of the rights of the participating source (developing) countries, including communities and indigenous peoples. While it was possible to develop guidelines for use in negotiating contracts and agreements, no single set of contractual terms could apply to all participants, and awardees have developed unique mechanisms and agreements to suit the particular circumstances of the organizations and countries involved (29) . As integrated conservation and development projects, the long term evaluation of this Program will depend on how successful the projects are in demonstrating the economic value of biodiversity in providing new pharmaceuticals and sustainable natural products-based industries for the participating developing countries.

Small Business Innovation Research (SBIR) and Small Business Technology Transfer (STTR) Programs. The SBIR program is a set-aside program designed to support innovative research by small U. S. business concerns (500 or less employees) that have the potential for commercialization of the subject of the research. The program is divided into two phases. Phase I covers a six-month period for feasibility studies of a proposed project, and currently can be funded to the extent of $100,000. Phase II covers a two-year period for development of any project considered of sufficient promise to clinical application and commercialization, and currently can be funded to the extent of $750,000. The STTR program supports cooperative research and development with potential for commercialization between small business concerns and U. S. non-profit research organizations.

The research topics should be in areas of emerging and high priority, and natural product topics of interest include:

- New biological methods for production of bioactive natural products.
- New systems for the large-scale production of active agents for preclinical and clinical development
- Newer methods for the isolation and purification of active agents.
- Methods for the isolation, purification, identification, cultivation, and extraction of microbes from unusual habitats.

Further information on the Small Business programs and funding opportunities throughout the National Institutes of Health may be obtained from the NIH homepage:

http://www.nih.gov/grants/funding/sbir.htm

New Directions in Natural Product Drug Discovery

Exploration of new environments. As has already been discussed, the potential of the marine environment as a source of novel drugs remains largely unexplored. Despite the more intensive investigation of terrestrial flora, it is estimated that only 5-15% of the approximately 250,000 species of higher plants have been systematically investigated, chemically and pharmacologically (30), and the potential of large areas of tropical rainforests remains virtually untapped.

The continuing threat to biodiversity through the destruction of terrestrial and marine ecosystems lends an urgency to the need to expand the exploration of these resources as a source of novel bioactive agents.

The unexplored potential of microbial diversity. Until recently, microbiologists were greatly limited in their study of natural microbial ecosystems due to an inability to cultivate most naturally occurring microorganisms. In a report recently released by the American Academy of Microbiology entitled "The Microbial World: Foundation of the Biosphere", it is estimated that "less than 1% of

bacterial species and less than 5% of fungal species are currently known", and recent evidence indicates that millions of microbial species remain undiscovered (31).

The recent development of procedures for cultivating and identifying microorganisms will aid microbiologists in their assessment of the earth's full range of microbial diversity. In addition, procedures based on the extraction of nucleic acids from environmental samples will permit the identification of microorganisms through the isolation and sequencing of ribosomal RNA or rDNA (genes encoding for rRNA); samples may be obtained from soils and marine habitats, as well as extreme habitats, such as hot springs, deep-sea vents, sea ice and polar lakes. Valuable products and information are certain to result from the cloning and understanding of the novel genes which will be discovered through these processes.

The report concludes that "these new microorganisms provide a vast untapped reservoir of genetic and metabolic diversity, the harvesting and study of which will have far-reaching, positive effects for society in areas such as enhanced food production, medicine (e.g., antibiotic discovery), bioremediation of waste materials, and agriculture" (31).

Targeting natural products. A recurring liability of natural products, at least in the area of cancer chemotherapy, is that although many are generally very potent, they have limited solubility in aqueous solvents and exhibit narrow therapeutic indices. These factors have resulted in the demise of a number of promising leads, such as bruceantin and maytansine.

An alternative approach to utilizing such agents is to investigate their potential as "warheads" attached to monoclonal antibodies specifically targeted to epitopes on tumors of interest (32). While this is not a new area of research to the NCI, the DTP is well established to refine and expand this approach to cancer therapy. The DTP has a wide range of potent, natural product chemotypes to explore as potential "warheads", and also has the capability to produce clinical grade monoclonal antibodies (Mabs) through its Biological Resources Branch. One of the approaches being investigated is the selection of a less potent member of a chemical class (e.g., ansamycin antibiotics) as the "warhead" in order to avoid undue toxic effects in the event of cleavage of the "warhead-Mab" bond prior to delivery of the agent to the desired target tumor cells. The potential for severe toxicity in such instances when using "warheads" of the potency of ricin or calicheamicin is substantial.

Another strategy of interest is the use of antibodies as vectors for enzymes capable of activating a nontoxic drug precursor (prodrug) to a potent cytotoxic moiety (33). After injection and localization of an antibody-enzyme conjugate at the tumor, a nontoxic prodrug is administered, and while remaining innocuous to the normal tissues, it is converted to the cytotoxin by the enzyme localized at the tumor. This approach, called "antibody-directed enzyme prodrug therapy" (ADEPT) provides further potential for the application of potent natural products to cancer treatment.

Combinatorial biosynthesis. Advances in the understanding of bacterial aromatic polyketide biosynthesis have lead to the identification of multifunctional polyketide synthase enzymes (PKSs) responsible for the construction of polyketide backbones of defined chain lengths, the degree and regio-specificity of ketoreduction, and the regiospecificity of cyclizations and aromatizations, together with the genes encoding for the enzymes (34). A set of rules for manipulating the early steps of aromatic polyketide biosynthesis through genetic engineering has been developed, permitting the biosynthesis of polyketides not generated naturally ("unnatural natural products"). Since polyketides constitute a large number of structurally-diverse natural products exhibiting a broad range of biological activities (e.g., tetracyclines, doxorubicin, and avermectin), the potential for generating novel molecules with enhanced known bioactivities, or even novel bioactivities, appears to be high.

The NCI is promoting this area of research through the award of grants to consortia composed of multidisciplinary groups devoted to the application of combinatorial biosynthetic and/or combinatorial chemical techniques to the generation of molecular diversity for testing in high throughput screens related to cancer.

Conclusion

As illustrated in the foregoing discussion, nature is an abundant source of novel chemotypes and pharmacophores. However, it has been estimated that only 5 to 15% of the approximately 250,000 species of higher plants have been systematically investigated for the presence of bioactive compounds (24), while the potential of the marine environment has barely been tapped (5). Tropical islands, such as Puerto Rico, offer excellent opportunities for the productive exploration of these resources. The *Actinomycetales* have been extensively investigated and have been, and remain, a major source of novel microbial metabolites (35); however, less than 1% of bacterial and less than 5% of fungal species are currently known, and the potential of novel microbial sources, particularly those found in extreme environments (31), seems unbounded. To these natural sources can be added the potential to investigate the rational design of novel structure types within certain classes of microbial

metabolites through genetic engineering, as has been elegantly demonstrated with bacterial polyketides (34). The proven natural product drug discovery track record, coupled with the continuing threat to biodiversity through the destruction of terrestrial and marine ecosystems, provides a compelling argument in favor of expanded exploration of nature as a source of novel anticancer agents.

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