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International collaboration in drug discovery and development: the NCI experience*

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Abstract: Over the millennia, natural products, mainly of plant origin, have been used for the treatment of diseases, and an impressive number of modern drugs have been isolated from natural sources based on their use in traditional medicine. The past century, however, has seen an increasing role played by micro-organisms in the production of antibiotics and other drugs for the treatment of diseases, ranging from bacterial infections to cardiovascular problems and cancer. The role of nature will continue to grow with the exploration of tropical rainforests and marine environments, as well as the huge untapped resource of micro-organisms which have, as yet, defied culture. With less than 1% of the microbial world currently known, the extraction of nucleic acids from environmental samples from soil and marine habitats, from symbiotic and endophytic microbes associated with terrestrial and marine macro-organisms, as well as from extreme habitats, such as hot springs and deep sea vents, will permit access to a vast reservoir of genetic and metabolic diversity. These resources will provide a host of novel chemical scaffolds which can be further developed by combinatorial chemical and biosynthetic approaches to yield chemotherapeutic and other bioactive agents which have been optimized on the basis of their biological activities.

MEDICINALS FOR THE MILLENNIA

Recorded history

Throughout 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 [1]. The first records, written on clay tablets in cuneiform, are from Mesopotamia and date from about 2600 BC; among the substances which they used were oils of *Cedrus* species (cedar) and *Cupressus sempevirens* (cypress), *Glycyrrhiza glabra* (licorice), *Commiphora* species (myrrh), and *Papaver somniferum* (poppy juice), all of which are still in use today for the treatment of ailments ranging from coughs and colds to parasitic infections and inflammation. Egyptian medicine dates from about 2900 BC, but the best known Egyptian pharmaceutical record is the 'Ebers Papyrus' dating from 1500 BC; this documents some 700 drugs (mostly plants), and includes formulas, such as gargles, snuffs, poultices, infusions, pills and ointments, with beer, milk, wine and honey being commonly used as vehicles. The

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Chinese Materia Medica has been extensively documented over the centuries, with the first record dating from about 1100 BC (Wu Shi Er Bing Fang, containing 52 prescriptions), followed by works such as the Shennong Herbal (≈100 BC; 365 drugs) and the Tang Herbal (659 AD; 850 drugs). Likewise, documentation of the Indian Ayurvedic system dates from about 1000 BC (Susruta and Charaka), and this system formed the basis for the primary text of Tibetan Medicine, Gyu-zhi (Four Tantras) translated from Sanskrit during the eighth century AD [2]. In the ancient Western world, the Greeks contributed substantially to the rational development of the use of herbal drugs. The philosopher and natural scientist, Theophrastus (~300 BC), in his *History of Plants*, dealt with the medicinal qualities of herbs, and noted the ability to change their characteristics through cultivation. Dioscorides, a Greek physician (100 AD), during his travels with Roman armies throughout the then 'known world', accurately recorded the collection, storage, and use of medicinal herbs, and is considered by many to be the most important representative of the science of herbal drugs in 'ancient times'. Galen (130–200 AD), who practiced and taught pharmacy and medicine in Rome, and published no less than 30 books on these subjects, is well known for his complex prescriptions and formulas used in compounding drugs, sometimes containing dozens of ingredients ('galenicals'). During the Dark and Middle Ages (fifth to twelfth centuries), the monasteries in countries such as England, Ireland, France and Germany, preserved the remnants of this Western knowledge, but it was the Arabs who were responsible for the preservation of much of the Greco-Roman expertise, and for expanding it to include the use of their own resources, together with Chinese and Indian herbs unknown to the Greco-Roman world. The Arabs were the first to establish privately owned drug stores in the eighth century, and the Persian pharmacist, physician, philosopher and poet, Avicenna, contributed much to the sciences of pharmacy and medicine through works, such as Canon Medicinae, regarded as 'the final codification of all Greco-Roman medicine'.

Traditional medicine and drug discovery

As mentioned above, plants have formed the basis for traditional medicine systems which have been used for thousands of years in countries such as China [3] and India [4]. The use of plants in the traditional medicine systems of many other cultures has been extensively documented [5–10]. 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 [11]. 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 [11]. 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.

The isolation of the anti-malarial drug quinine from the bark of Cinchona species (e.g. *C. officinalis*) was reported in 1820 by the French pharmacists, Caventou and Pelletier. The bark had long been used by indigenous groups in the Amazon region for the treatment of fevers, and was first introduced into Europe in the early 1600s for the treatment of malaria. Quinine formed the basis for the synthesis of the commonly used anti-malarial drugs, chloroquine and mefloquine. With the emergence of resistance to these drugs in many tropical regions, another plant long used in the treatment of fevers in traditional Chinese medicine, *Artemisia annua* (Quinhaosu), has yielded the agents, artemisinin (Fig. 1) and its derivatives, artemether and artether, effective against resistant strains [12]. The analgesic, morphine, isolated in 1816 by the German pharmacist, Serturner, from the opium poppy, *Papaver somniferum*, used in ancient Mesopotamia (see below), laid the basis for alkaloid chemistry, and the development of a range of highly effective analgesic agents [12]. In 1785, the English physician, Withering, published his observations on the use of the foxglove, *Digitalis purpurea*, for the treatment of heart disorders, and this eventually led to the isolation of the cardiotonic agent, digoxin.

Other significant drugs developed from traditional medicinal plants include: the anti-hypertensive agent, reserpine, isolated from *Rauwolfia serpentina* used in Ayurvedic medicine for the treatment of snakebite and other ailments [4]; ephedrine, first isolated in 1887 from *Ephedra sinica* (Ma Huang), a plant long used in traditional Chinese medicine, and basis for the synthesis of the anti-asthma agents (beta

agonists), salbutamol and salmetrol [12]; and the muscle relaxant, tubocurarine, isolated from *Chondrodendron* and *Curarea* species used by indigenous groups in the Amazon as the basis for the arrow poison, curare [12].

The golden age of antibiotics

The serendipitous discovery of penicillin from the filamentous fungus, *Penicillium notatum*, by Fleming in 1929, and the observation of 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. Micro-organisms are a prolific source of structurally diverse bioactive metabolites and have yielded some of the most important products of the pharmaceutical industry. These include: anti-bacterial agents, such as the penicillins (from *Penicillium* species), cephalosporins (from *Cephalosporium acremonium*), aminoglycosides, tetracyclines and polyketides (all from *Streptomyces* species) [12]; immunosuppressive agents, such as the cyclosporins and rapamycin (from *Streptomyces* species) [13]; cholesterol lowering agents, such as mevastatin (compactin) and lovastatin (from *Penicillium* species) [12]; and anthelmintics and anti-parasitic drugs, such as the ivermectins (from *Streptomyces* species) [12]. A recent publication reports the isolation of a potential anti-diabetic agent from a *Pseudomassaria* fungal species found in the rainforests of the Congo [14].

Marine sources

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 woollen 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 [15].

Prior to the development of reliable scuba diving techniques some 40 years ago, the collection of marine organisms was limited to those obtainable by 'skin diving'. Subsequently, depths from approximately 10 feet to 120 feet (3–35 m) became routinely attainable, and the marine environment has been increasingly explored as a source of novel bioactive agents. Deep water collections can be made by dredging or trawling, but these methods suffer from disadvantages, such as environmental damage and nonselective sampling. These disadvantages can be partially overcome by use of manned submersibles or remotely operated vehicles (ROVs), however, the high cost of these forms of collecting precludes their extensive use in routine collection operations.

The pseudopterosins, isolated from the Carribbean gorgonian, *Pseudopterogorgia elisabethae*, possess significant analgesic and anti-inflammatory activity, and defined fractions obtained from extracts of the gorgonian are used topically in skin lotions. Another marine product showing potent anti-inflammatory activity, manoalide, isolated from the sponge, *Luffarriella variabilis* [15], has led to a family of similar compounds via synthesis, some of which have reached clinical trial status. The extremely potent venoms (conatoxins) of predatory cone snails (*Conus* species) have yielded complex mixtures of small peptides (6–40 amino acids) which have provided models for the synthesis of novel painkillers (e.g. Ziconotide) [16].

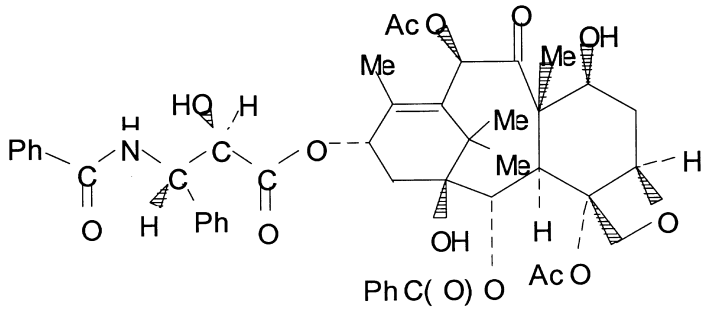
Other sources

Teprotide, isolated from the venom of the pit viper, *Bothrops jaracaca*, led to the design and synthesis of the ACE inhibitors, captropril and enalapril [12], used in the treatment of cardiovascular disease, while epibatidine, isolated from the skin of the poisonous frog, *Epipedobates tricolor*, has led to the development of a novel class of painkillers [17].

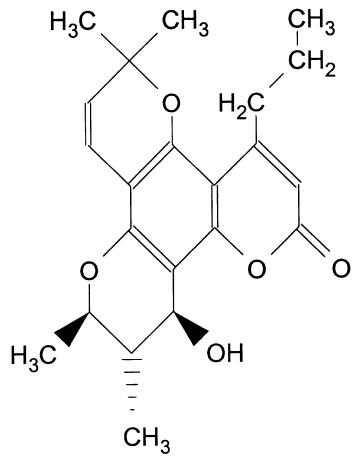
This interest in nature as a source of potential chemotherapeutic agents continues, and an analysis of the number and sources of anti-cancer and anti-infective agents, reported mainly in the Annual Reports of Medicinal Chemistry from 1984 to 1995 covering the years 1983–94, indicates that over 60% of the approved drugs developed in these disease areas are of natural origin [18].

Some examples of natural products that are used as drugs, or that can be modified to produce drugs with improved pharmacological characteristics are given in Fig. 1.

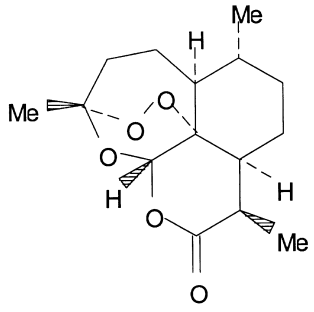
Plant Products



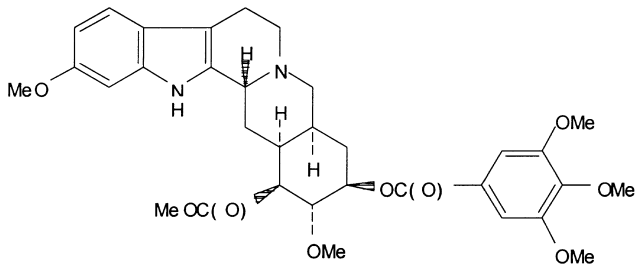
Paclitaxel



Calanolide A

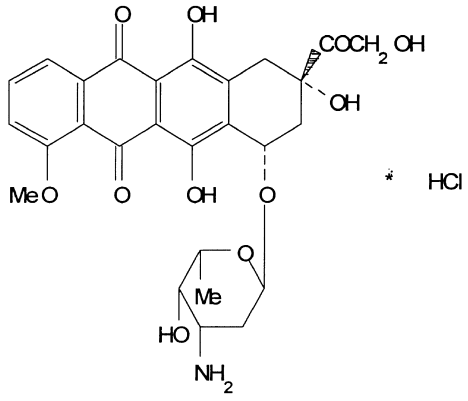


Artemisinin

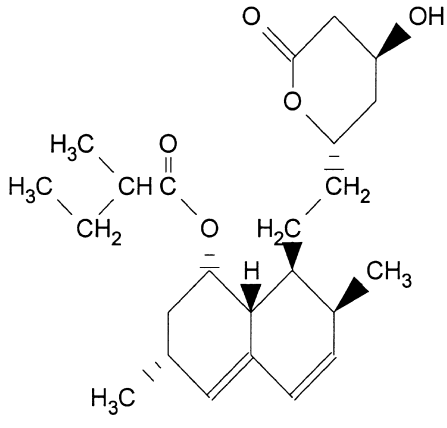


Reserpine

Microbial Products



Doxorubicin (Adriamycin)



Mevastatin

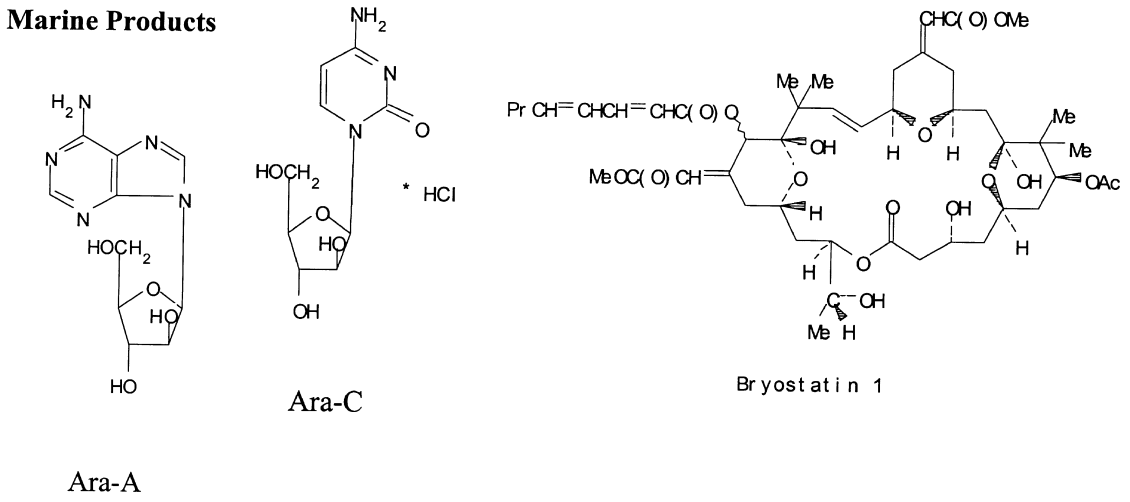


Fig. 1 Natural products that are used as drugs or as sources of drugs.

ANTICANCER AGENTS DERIVED FROM NATURAL SOURCES

Of the 92 anti-cancer drugs commercially available prior to 1983 in the USA and approved world-wide between 1983 and 1994, approximately 62% can be related to natural origin [18].

Plant sources

Plants have a long history of use in the treatment of cancer [19], though many of the claims for the efficacy of such treatment should be viewed with some scepticism because cancer, as a specific disease entity, is likely to be poorly defined in terms of folklore and traditional medicine [20]. Of the plant-derived anti-cancer drugs in clinical use, the best known are the so-called vinca alkaloids, vinblastine and vincristine, isolated from the Madagascar periwinkle, *Catharanthus roseus*. *C. 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 [20]. The two clinically active agents, etoposide and teniposide, which are semisynthetic 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 anti-tumor 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 [20].

More recent additions to the armamentarium of naturally derived chemotherapeutic agents are the taxanes and camptothecins. Paclitaxel (Fig. 1) initially was isolated from the bark of *Taxus brevifolia*, collected in Washington State as part of a random collection program by the U.S. Department of Agriculture for the National Cancer Institute (NCI) [21]. The use of various parts of *T. brevifolia* and other *Taxus* species (e.g. *canadensis*, *baccata*) by several Native American tribes for the treatment of some noncancerous conditions has been reported [20], while the leaves of *T. baccata* are used in the traditional Asiatic Indian (Ayurvedic) medicine system [4], with one reported use in the treatment of 'cancer' [19]. Paclitaxel, along with several key precursors (the baccatins), occurs in the leaves of various *Taxus* species, and the ready semisynthetic conversion of the relatively abundant baccatins to paclitaxel, as well as active paclitaxel analogs, such as docetaxel [22], has provided a major, renewable natural source of this important class of drugs. Likewise, the clinically active agents, topotecan (hycamptamine), irinotecan (CPT-11), 9-amino-and 9-nitro-camptothecin, are semisynthetically derived from camptothecin, isolated from the Chinese ornamental tree, *Camptotheca acuminata* [23]. 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.), and elliptinium, a derivative of ellipticine, isolated from species of several genera of the *Apocynaceae* family, including *Bleekeria vitensis*, a Fijian medicinal plant with reputed anti-cancer properties [20]. Homoharringtonine has shown efficacy against various leukemias, while elliptinium is marketed in France for the treatment of breast cancer [24]. The flavone, flavopiridol, currently in Phase I clinical trials, is scheduled to be advanced to Phase II trials against a broad range of tumors [25]. While flavopiridol is totally synthetic, the basis for its novel structure is a natural product isolated from *Dysoxylum binectariferum* [26]. Ipomeanol, a pneumotoxic furan derivative produced by sweet potatoes (*Ipomoea batatas*) infected with the fungus, *Fusarium solani,* has been in clinical trials for treatment of lung cancer [24].

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 [24].

Microbial sources

Antitumor antibiotics are amongst the most important of the cancer chemotherapeutic agents, which include members of the anthracycline, bleomycin, actinomycin, mitomycin and aureolic acid families [27]. Clinically useful agents from these families are the daunomycin-related agents, daunomycin itself, doxorubicin (Fig. 1), idarubicin and epirubicin; the glycopeptidic bleomycins A_2 and B_2 (blenoxane); the peptolides exemplified by dactinomycin; the mitosanes such as mitomycin C; and the glycosylated anthracenone, mithramycin. All were isolated from various *Streptomyces* species. Other clinically active agents isolated from *Streptomyces* include streptozocin and deoxycoformycin.

Microbial metabolites in past or present clinical trials include acivicin, aclacinomycin, deoxyspergualin, echinomycin, elsametrocin, fostriecin, menogaril, porfiromycin, quinocarmycin and rhizoxin, as well as the glycinate of aphidicolin. Microbial products predominate amongst the agents under development by the Division of Cancer Treatment and Diagnosis (DCTD) of the NCI [25]. These include UCN-01 (7-hydroxystaurosporine), isolated from a *Streptomyces* species, and FR901228, a novel bicyclic depsipeptide isolated from a *Chromobacterium violaceum* strain, as well as derivatives of quinocarmycin (DX-52-1), spicamycin (KRN5500), CC-1065 (bizelesin), tetracycline (COL-3), rapamycin, and rebeccamycin. Recent exciting discoveries are the epothilones isolated from myxobacteria [28]. This class of compounds has been shown to act by a similar mechanism of action to paclitaxel and could complement the taxanes as chemotherapeutic agents.

The large number of microbial agents reflects the major role played by the pharmaceutical industry in this area of drug discovery and development. Generally, industry has focused on the *Actinomycetales*, but expansion of research efforts, often supported by government funding, to the study of organisms from diverse environments, such as shallow and deep marine ecosystems and deep terrestrial subsurface layers, has demonstrated their potential as a source of novel bioactive metabolites [29].

Marine sources

The first notable discovery of biologically active compounds from marine sources was the serendipitous isolation of the C-nucleosides, spongouridine and spongothymidine, from the Caribbean sponge, *Cryptotheca crypta*, in the early 1950s. These compounds were found to possess anti-viral activity, and synthetic analog studies eventually led to the development of cytosine arabinoside (Ara-C) as a clinically useful anti-cancer agent approximately 15 years later [15], together with Ara-A as an anti-viral agent (Fig. 1). The systematic investigation of marine environments as sources of novel biologically active agents only began in earnest in the mid-1970s. During the decade from 1977 to 1987, about 2500 new metabolites were reported from a variety of marine organisms. These studies have clearly demonstrated that the marine environment is a rich source of bioactive compounds, many of which belong to totally novel chemical classes not found in terrestrial sources [30].

As yet, no compound isolated from a marine source has advanced to commercial use as a chemotherapeutic agent, though several are in various phases of clinical development as potential anti-cancer agents. The

most prominent of these is bryostatin 1 (Fig. 1), isolated from the bryozoan, *Bugula neritina* [15]. This agent exerts a range of biological effects, thought to occur through modulation of protein kinase C, and has shown some promising activity against melanoma in Phase I studies [31]. Phase II trials are either in progress or are planned against a variety of tumors, including ovarian carcinoma and NHL.

The first marine-derived compound to enter clinical trials was didemnin B, isolated from the tunicate, *Tridemnum solidum* [15]. Unfortunately, it has failed to show reproducible activity against a range of tumors in Phase II clinical trials, while always demonstrating significant toxicity. Ecteinascidin 743, a metabolite produced by another tunicate *Ecteinascidia turbinata*, has significant *in vivo* activity against the murine B16 melanoma and human MX-1 breast carcinoma models, and currently is scheduled for Phase II clinical trials in Europe and the United States (personal communication, G. Faircloth, PharmaMar). The sea hare, *Dolabella auricularia*, an herbivorous mollusc from the Indian Ocean, is the source of more than 15 cytotoxic cyclic and linear peptides, the dolastatins. The most active of these is the linear tetrapeptide, dolastatin 10, which has been chemically synthesized and is currently in Phase I clinical trials [30]. Sponges are traditionally a rich source of bioactive compounds in a variety of pharmacological screens [30], and in the cancer area, halichondrin B, a macrocyclic polyether initially isolated from the sponge, *Halichondria okadai* in 1985, is currently in preclinical development by the NCI. Halichondrin B and related compounds have been isolated from several sponge genera, and the present source, a *Lissodendoryx* species, is being successfully grown by in-sea aquaculture in New Zealand territorial waters [32]. The mechanisms of action of discodermolide [33], isolated from the Caribbean sponge, *Discodermia* sp., and eleutherobin [34], isolated from a Western Australian soft coral, *Eleutherobia* sp., are similar to that of paclitaxel, with the former now in preclinical development with Novartis.

CURRENT STATUS OF THE NCI NATURAL PRODUCTS DRUG DISCOVERY PROGRAM

Drug discovery

The 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 DCTD. Thus, NCI has for the past 40 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, world-wide, and has played a major role in the discovery and development of many of the available commercial and investigational anti-cancer agents. During this period, more than 400 000 chemicals, both synthetic and natural, have been screened for anti-tumor 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 chemical structures with a wide variety of biological activities. From 1960 to 1982 over 180 000 microbial-derived, some 16 000 marine organism-derived, and over 114 000 plant-derived extracts were screened for anti-tumor activity, mainly by the NCI, and, as illustrated above, a number of clinically effective chemotherapeutic agents have been developed. Most of the drugs currently available for cancer therapy, however, are effective predominantly against rapidly proliferating tumors, such as leukemias and lymphomas, but (with some notable exceptions such as the camptothecins, doxorubicin and the taxanes), show little useful activity against the slow-growing solid tumors usually associated with adults, such as lung, colon, prostatic, pancreatic, and brain tumors. In the early 1980s, the NCI collection 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 could, 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 (a rapidly growing tumor line)

screen appeared to be detecting only previously identified active compounds or chemical structure types having little or no activity against slow-growing solid tumors.

During 1985–90, the NCI developed a new *in vitro* primary screen based upon a diverse panel of human tumor cell lines [35]. The screen currently comprises 60 cell lines derived from nine cancer types, and organized into subpanels representing leukemia, lung, colon, central nervous system, melanoma, ovarian, renal, prostate and breast. In early 1999, a preliminary prescreen involving single high-dose testing of materials against three cell lines [MCF-7 (breast), H-460 (lung), and SF-268 (CNS)] was introduced, and those materials showing significant activity in one or more of the three lines are advanced to the 60 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 anti-cancer 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 based in Palau in Micronesia. Terrestrial plant collections have been carried out in over 25 countries in tropical and subtropical regions world-wide 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 (South-east 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 1–12 months to participate in collaborative natural products research. Representatives of most of the source countries have visited the NCI and contractor facilities for shorter periods to discuss collaboration [36]. Contract collections of plants are now being de-emphasized in favor of establishing direct collaborations with qualified organizations in the source countries (discussed below).

Dried plant samples (0.3–1 kg dry weight) and frozen marine organism samples ($\approx$ 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 extracts are assigned discreet NCI 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.

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 [37]. 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) [38], and from 1988 to 1996, over 90 000 extracts were tested in this screen. In late 1996, the screening of extracts was discontinued, and alternative assays involving the use of targeted enzyme systems are now being used.

Pre-clinical development

Those agents showing significant *in vivo* activity are presented to the NCI Division of Cancer Treatment and Diagnosis (DCTD) Decision Network Committee (DNC), and, if approved by the DNC, the agent is entered into preclinical and clinical development. The Decision Network Process divides the preclinical drug development process into stages designated as DNIIA, DNIIB, and DNIII depending upon the level of resources required. These are equivalent to what the pharmaceutical industry would define as early preclinical stage, advanced preclinical stage and clinical candidate. The requirements for a natural product-derived compound are as described below.

- An adequate supply of natural product is procured to permit preclinical and clinical development.
- Formulation studies are performed to develop a suitable vehicle to solubilize the drug for 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 determines the best route and schedule of administration to achieve optimal activity of the drug in animal models, the half-lives and bio-availability 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, IND-directed 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.

Alternatively, agents may be developed through RAID (Rapid Access to Intervention Development), 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 drug, and functions as a collaboration between the NCI and the originating laboratory, with tasks 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 discussed above, the products of the RAID program are returned directly to the originating laboratory for proof-of-principle clinical trials. 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.

Clinical development

Phase I studies are conducted to determine the maximum tolerated dose (MTD) of a drug in humans and to observe the sites and reversibilities of any toxic effects. In contrast to trials with agents directed at other diseases, all patients in Phase I cancer trials have some form of the disease. 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 generally are 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.

Of the 54 anti-cancer agents currently in active preclinical or Phase I development by NCI (excluding biologics), 29 are either the natural product, derived from a natural product, or are synthetic with the structure based upon a natural product. The source organisms that these compounds were either derived from or based upon are 13 microbial, 3 marine, 6 plant, and 7 animal. The rest of the agents [25] are synthetic chemicals, not based on natural product structures.

COLLABORATION IN DRUG DISCOVERY AND DEVELOPMENT: THE NCI ROLE

Much of the NCI drug discovery and development effort has been, and continues to be, carried out through collaborations with academic institutions, research organizations and the pharmaceutical industry world-wide. Many of the naturally derived anti-cancer agents were developed through such efforts. The DTP/NCI thus complements the efforts of the pharmaceutical industry and other research organizations through taking positive leads, which industry might consider too uncertain to sponsor, and conducting the 'high risk' research necessary to determine their potential utility as anti-cancer drugs. In promoting drug discovery and development, the DTP/NCI has formulated various mechanisms for establishing collaborations with research groups world-wide.

Source country collaboration: drug discovery: memorandum of understanding

As discussed, 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 drug discovery program [36].

With the increased awareness of genetically rich source countries to the 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 [39]. 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, the 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 [39]. Through this mechanism collaborations have been established with organizations in Bangladesh, Brazil, China, Costa Rica, Fiji, Iceland, Korea, Mexico, New Zealand, Nicaragua, Pakistan, Panama, South Africa, and Zimbabwe.

Drug development: the calanolides

In 1988, an organic extract of the leaves and twigs of the tree, *Calophyllum lanigerum*, collected in Sarawak, Malaysia 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 (Fig. 1) as the main *in vitro* active agent [40]. 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 showing significant anti-HIV activity. The active constituent was found to be (–)-calanolide B which was isolated in yields of 20–30%. 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. A decision was made by the NCI/DNC to proceed with the preclinical development of both 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 their development

was awarded to Medichem Research, Inc., a small pharmaceutical company based near Chicago. Medichem Research had developed a synthesis of (+)-calanolide A [41] 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. 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. Medichem Research, in collaboration with the NCI through the signing of a Cooperative Research and Development Agreement (CRADA) by which NCI is contributing research knowledge and expertise, has advanced (+)-calanolide A through preclinical development, and was granted an INDA for clinical studies by the USA Food and Drug Administration (FDA). The Sarawak State Government and Medichem Research formed a joint venture company, Sarawak Medichem Pharmaceuticals Incorporated (SMP), in late 1996, and SMP has sponsored Phase I clinical studies with healthy volunteers. It has been shown that doses exceeding the expected levels required for efficacy against the virus are well tolerated. Trials using patients infected with HIV-1 were initiated in early 1999.

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 [42].

Distribution of extracts from the NCI natural products repository

In carrying out the collection and extraction of thousands of plant and marine organism samples world-wide, 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) which protects the rights of all parties (see DTP WWW Homepage below). One of the key terms of the MTA is the requirement that the recipient organization negotiate suitable terms of collaboration and compensation with the source country(ies) of any extract(s) which are developed towards clinical trials and possible commercialization.

DTP WWW homepage

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

Publicly available data include results from the human tumor cell line screen and AIDS anti-viral 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 [35], the AIDS anti-viral drug screen, and the 3D database. 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, they 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.

Screening agreement

In the case of organizations wishing to have pure compounds tested in the NCI drug screening program, such as pharmaceutical and chemical companies or academic research groups, the DTP/NCI has formulated a screening agreement which includes terms stipulating confidentiality, patent rights, routine and nonproprietary screening and testing vs. nonroutine, 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. Should a compound show promising anti-cancer 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).

Cooperative natural product drug discovery programs

In order to foster multidisciplinary research, the NCI introduced the National Cooperative Natural Product Drug Discovery Group Program (NCNPDDG) in the late 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 from natural sources [43]. In the early 1990s, the International Cooperative Biodiversity Group Program (ICBG), similar to the NCNPDDG Program, but also requiring participation by developing source countries, was initiated with joint sponsorship from several US Government agencies. In addition to drug discovery, the ICBG emphasizes the inventory and conservation of natural resources, as well as the economic development of the participating source countries. The structure of these programs is summarized in Table 1. The inclusion of an industrial component in almost all consortia has had 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 Programs. The participation by developing source countries in the ICBG, as well as in most of the NCNPDDG programs, has contributed to the training of source country scientists and technology transfer, as well as the inventory and conservation of biodiversity. Source countries representing most major ecosystems are participating, and agreements protecting the rights of all parties have been developed [44].

Table 1 Types of cooperative drug discovery groups

Content/Type	ICBG	NCNPDDG
Source country involvement	Yes	Yes
Access to NCI/NPR	Yes	Yes
US funding agencies	NIH, NSF, USDA	NCI
Academia	Yes	Yes
Industry	Yes	Yes
US govt. scientists	Yes	Yes
Source country scientists	Yes	Yes
Source country regions	South and Central America West Africa and Madagascar South-east Asia	South and Central America Africa and Madagascar South-east Asia
IPR agreements	Yes	Yes

NEW DIRECTIONS IN NATURAL PRODUCT DRUG DISCOVERY

Exploration of new environments

As 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 [45], 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 exploration of these resources as a source of novel bioactive agents.

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 micro-organisms. 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 [29,46].

New development of procedures for cultivating and identifying micro-organisms 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 micro-organisms through the isolation and sequencing of ribosomal RNA or rDNA (genes encoding for rRNA). Samples may be obtained from soils and marine habitats, as symbiotic or endophytic microbes associated with terrestrial or marine macro-organisms, as well as from 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 micro-organisms 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' [46].

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 [47]. 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.

CONCLUSION

As illustrated in the foregoing discussion, nature is an abundant source of novel chemotypes and pharmacophores. However, it has been estimated that only 5–15% of the approximately 250 000 species of higher plants have been systematically investigated for the presence of bioactive compounds [45], while the potential of the marine environment has barely been tapped [15]. The *Actinomycetales* have been extensively investigated and have been, and remain, a major source of novel microbial metabolites [48]; 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 [45], 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 [47]. 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 leads for the development of drugs and other valuable bioactive agents. It is apparent that nature can provide the novel chemical scaffolds for elaboration by combinatorial approaches (chemical and biochemical), thus leading to agents that have been optimized on the basis of their biological activities.

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