International Organization for Chemical Sciences in Development

Working Group on Plant Chemistry

CHEMISTRY, BIOLOGICAL AND PHARMACOLOGICAL PROPERTIES OF AFRICAN MEDICINAL PLANTS

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Edited by

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3. International collaboration in drug discovery and development. The United States National Cancer Institute experience

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Introduction

In 1937, the United States National Cancer Institute (NCI) was established with its mission being "to provide for, foster and aid in coordinating research related to cancer". The NCI is the largest of seventeen Institutes which comprise the National Institutes of Health (NIH), which are components of the Federal Government's Department of Health and Human Services. The NCI and NIH are entirely funded through appropriations from the U.S. Congress and, as such, are entirely non-commercial and non-profit. Thus, while the NCI will attempt to license drugs discovered through its screening programs to pharmaceutical companies for advanced development and marketing, chemotherapeutic agents not licensed, but considered by NCI to be clinically effective, will be distributed by the NCI at no cost to the patients. An example is the antileukemic agent, Erwinia L-asparaginase, which was procured by the NCI and distributed free of charge until its production and marketing were transferred to the private sector.

In 1955, NCI set up the Cancer Chemotherapy National Service Center (CCNSC) to promote a cancer chemotherapy program, involving the procurement, screening, preclinical development, and clinical evaluation of new agents. All aspects of drug discovery and preclinical development are now the responsibility of the Developmental Therapeutics Program (DTP), a major component of the Division of Cancer Treatment, Diagnosis and Centers (DCTDC). During the past 40 years, over 300,000 chemicals submitted by investigators and organizations worldwide, have been screened for antitumor activity, and NCI has played a major role in the discovery and development of almost all of the available commercial and investigational anticancer agents (Boyd 1993).

Naturally-derived anticancer agents

From 1960 to 1982, over 180,000 microbial fermentation products, and over 114,000 plant-derived and 16,000 marine organism 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 of animal tumor models and human tumor xenografts. Those agents showing significant activity were assigned priorities for preclinical and clinical development.

Much of the drug discovery effort was carried out through collaborations with research organizations and the pharmaceutical industry, which either submitted compounds on a voluntary basis or were supported by NCI through contract or grant funding mechanisms. A large number of novel agents belonging to a wide variety of chemical classes were isolated and characterized (Cassady and Douros 1979), but few of these new agents satisfied the stringent preclinical development requirements and advanced to clinical trials (Cragg *et al.* 1993a). Commercial agents of microbial origin include actinomycin D, bleomycin and doxorubicin (adriamycin) (Fig. 3.2), while plant-derived commercial drugs include vinblastine, vincristine, etoposide and teniposide (semisynthetic derivatives of epipodophyllotoxin, an epimer of podophyllotoxin), and taxol (Fig. 3.3). While no marine organism-derived agent has yet been approved for commercial use, bryostatin isolated from the bryozoan, *Bugula neritina*, is showing promise in clinical trials (Fig. 3.4) (Philip *et al.* 1993). Over ten natural product agents are in various stages of clinical development and, of these, the semisynthetic derivatives of camptothecin, irenotecan (CPT-11), topotecan and 9-aminocamptothecin (Fig. 3.5), are showing promising clinical activity against a variety of cancer disease types (Wall and Wani 1993). Over 60% of the compounds currently in preclinical and clinical development by NCI are of natural product origin (Fig. 3.1).

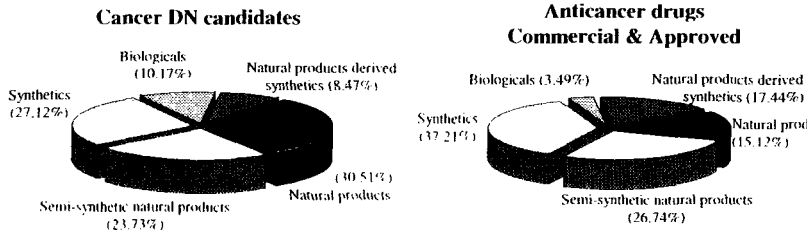
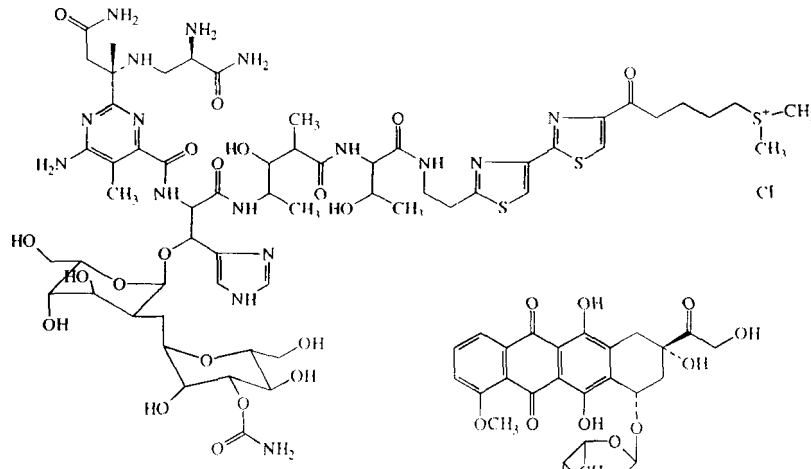
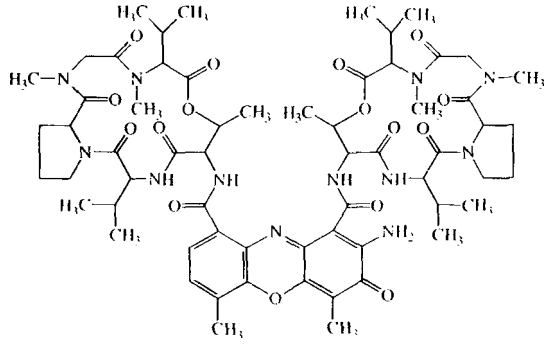


Fig. 3.1. Natural products in drug discovery.



Bleomycin A2
NSC-125066

Adriamycin hydrochloride
NSC-123127

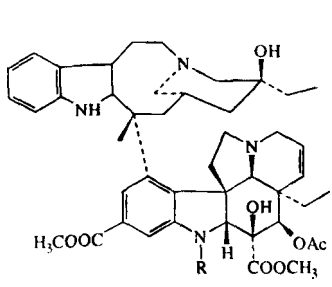


Actinomycin D
NSC-3053

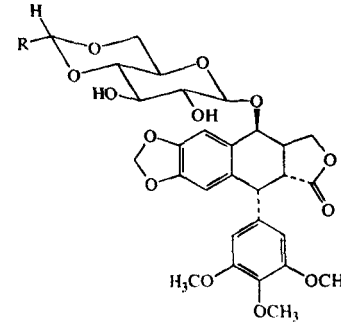
Fig. 3.2. Commercial agents of microbial origin.

Drug discovery at the NCI: Current status

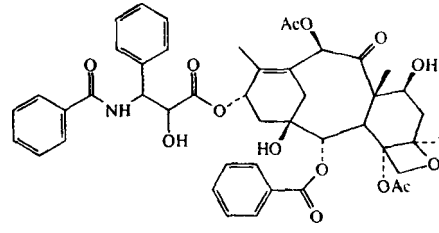
While the primary focus of the NCI drug discovery program remains the search for new anticancer agents, in 1987 the mission of the NCI was expanded to



Vincristine: R = CHO
Vinblastine: R = CH_3



Etoposide: R = CH_3
Teniposide: R =



Taxol

Fig. 3.3. Plant-derived commercial drugs.

include the discovery and preclinical development of agents for the treatment of acquired immunodeficiency syndrome (AIDS) (Boyd 1988).

The NCI places a major emphasis on natural products as a source of potential new anticancer and anti-HIV agents, and continues to investigate all natural sources. A contract for the cultivation and extraction of fungi is being performed through a contract with Science Applications International Corporation (SAIC) in NCI facilities at Frederick, Maryland, while the collection of marine invertebrates is being carried out in the Indo-Pacific region through a contract with Coral Reef Research Foundation (CRRF). Collections have been concentrated in the south western Pacific Ocean, but negotiations for expanding the collection program to Madagascar and Tanzania are currently in progress. Terrestrial plant collections have been carried out in over 25 countries in tropical and subtropical regions worldwide through contracts with Missouri Botanical Garden (Africa and Madagascar), New York Botanical Garden (Central and South America), and the University of Illinois at Chicago (Southeast Asia).

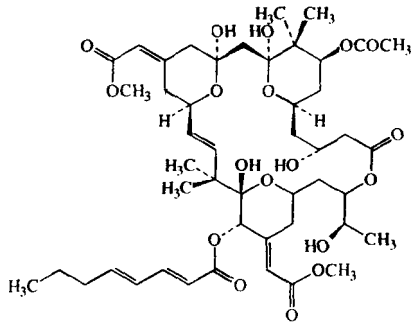


Fig. 3.4. Bryostatin.

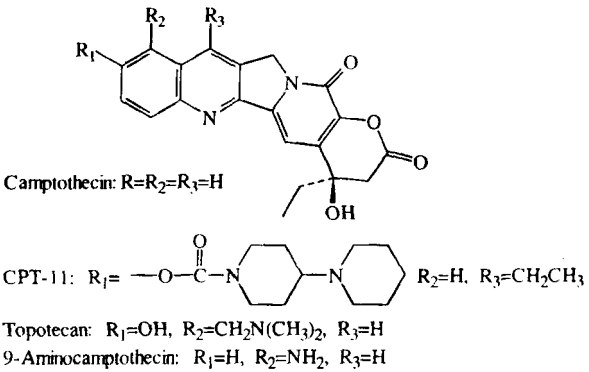


Fig. 3.5. Semisynthetic derivatives.

In carrying out these collections, the NCI contractors work closely with qualified organizations in each of the source countries. Organizations which have collaborated with the NCI contractor in Africa and Madagascar are listed in Table 3. 1.

Table 3.1. Plant Collection Collaborations in Africa and Madagascar

Cameroon	University of Yaounde; Committee for the Follow-up of the exploration and protection of *Ancistrocladus korupensis*
Gabon	Centre National de la Recherche Scientifique et Technologique
Ghana	University of Ghana, Legon; Ghana Herbarium
Madagascar	Centre National d'Application des Recherches Pharmaceutiques
Tanzania	Institute of Traditional Medicine, Muhimbili University College of Health Sciences.

To date, botanists and marine biologists from source country organizations have collaborated in field collection activities and taxonomic identifications, and their knowledge of local species and conditions has been indispensable to the success of the NCI collection operations. Source country organizations provide facilities for the preparation, packaging, and shipment of the samples to the NCI natural products repository in Frederick, Maryland. The collaboration between the source country organizations and the NCI collection contractors has, in turn, provided support for expanded research activities by source country biologists, and the deposition of a voucher specimen of each species collected in the national herbarium or repository is expanding source country holdings of their biota. When requested, NCI contractors also provide training opportunities for local personnel through conducting of 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, to participate in collaborative natural products research. To date, scientists (usually chemists) from all the countries listed in Table 3. 1, with the exception of Gabon, have carried out joint research projects for 3-12 months in U.S. laboratories, sponsored by NCI, while representatives of each country have visited NCI and Missouri Botanical Garden for shorter periods.

Dried plant samples (0.3-1kg dry weight) and frozen marine organism samples are shipped to the NCI Natural Products Repository (NPR) in Frederick where they are stored at -20°C prior to extraction with a 1:1 mixture of methanol: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.

Extracts are tested *in vitro* for selective cytotoxicity against panels of human cancer cell lines representing major disease types, including leukemia, melanoma, lung, breast, colon, central nervous system, ovarian, prostate and renal cancers (Boyd 1989). *In vitro* anti-AIDS activity is determined by measuring the survival of virus-infected human lymphoblastoid cells in the presence or absence of the extracts (Boyd 1988). Extracts showing significant selective cytotoxicity or anti-AIDS activity are subjected to bioassay-guided fractionation by chemists and biologists to isolate the pure chemical constituents responsible for the observed activity (Fig. 3.6). Bioassay-guided fractionation is essential since, in most instances, the active constituents are present in only small amounts in the crude extracts, and are generally isolated in yields of 0.01% or less, based on the mass of raw material. After the active constituent is isolated from an extract, its complete chemical structure is elucidated using modern spectroscopic techniques, and, if necessary and possible, X-ray crystallography.

This isolation and structural elucidation of a potential new agent is but the first phase in a lengthy process of development towards clinical trials, and possible general clinical use.

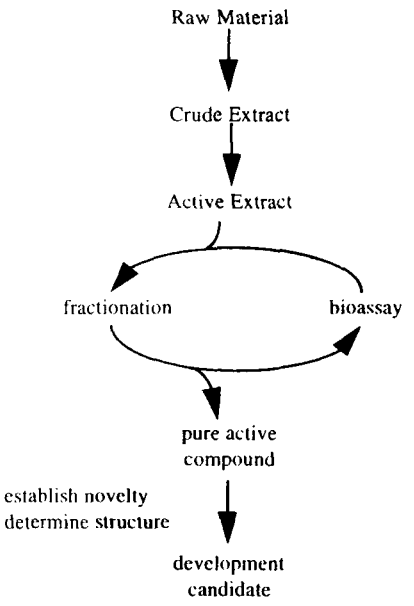


Fig. 3.6. Discovery of new natural products.

Drug development

Agents showing significant activity in the primary *in vitro* human cancer cell line or anti-AIDS screens are entered into various stages of preclinical development to determine their suitability for eventual advancement to clinical trials with human patients.

Preclinical development

Large-scale production of natural products - The initial plant sample (0.3-1.0 kg) collected by the contractor generally yields 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, while approval for clinical development could require multi-kilogram quantities.

To obtain sufficient quantities of an active agent for preclinical development, recollections of 5 to 100 kg of dried plant material, preferably from the original collection location, might be necessary; considerably larger quantities (sometimes exceeding 1000 kg) would be required for subsequent clinical development. The performance of large recollections necessitates surveys of the distribution and abundance of the plant, as well as determination of the variation of drug content in

different plant parts and the fluctuation of content with the season of harvesting. The potential for mass cultivation of the plant would also need to be assessed. If problems are encountered due to scarcity of the wild plant 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 plant tissue culture, can be investigated. While total synthesis must always be considered as a potential route for bulk production of the active agent, it should be noted that the structures of most bioactive natural products are extremely complex, and laboratory bench-scale syntheses often are not readily adapted to large-scale economic production.

The investigation of methods for the large-scale production of an active agent needs to be initiated well before it can be determined whether or not it will become a successful drug. Failure to address this issue could result in a supply crisis should an agent prove to be effective in clinical trials and advance to commercial use. The preclinical development of the *in vitro* active anti-HIV agent, michellamine B (see Section 5), illustrates this strategy.

Formulation - Formulation studies involve the development of 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-product agents in water poses considerable formulation problems, but these can often be overcome by use of co-solvents or emulsifying agents (surfactants) (Davignon and Craddock 1987). In the case of the anticancer drug, taxol, a vehicle was developed using ethanol and the emulsifying agent, Cremophore EL (polyoxyethylated castor oil), which is diluted with saline solution prior to administration to patients. Cremophore EL, however, elicits a strong allergic reaction from some patients, and considerable care has to be exercised in the intravenous administration of the solubilized drug.

Pharmacological evaluation - Pharmacological evaluation involves the study of various drug parameters and properties in suitable animal models. Data are developed to determine the route and schedule of administration which give optimal activity. Sensitive, selective analytical techniques are developed to enable the quantitative determination of the drug and its metabolites in biological fluids, such as blood, plasma, and urine; analytical methods used during the chemical isolation and purification procedures might not be suitable, since the compounds of interest often only occur in trace quantities in the biological fluids. Pharmacokinetic studies determine the half-lives, bioavailability, and effective concentrations of the drugs in blood and plasma, as well as their rates of clearance, excretion routes and metabolism. The identity and rates of formation of metabolites can provide insight into the possible mechanisms of action whereby the drugs exert their therapeutic and toxicological effects (Cragg and Suffness 1988). Pharmacokinetic and metabolism data can be used to design analogs and congeners of lead compounds with the aim of enhancing activity and/or decreasing toxicity.

Animal studies with taxol determined that the periodic administration of the drug in small quantities provided greater efficacy in the treatment of various tumors compared to administration of a single large dose (bolus injection); these observations have been confirmed in clinical studies, where a slow infusion over several hours has proved to be more effective in terms of tumor regression and reduction of allergic reactions from the patients.

Toxicological evaluation - Detailed toxicological evaluation is required to determine the type and degree of major toxicities in rodent and dog models, and to develop data for the determination of safe starting doses in humans. Studies are designed to determine the relationship of toxicity to dose and schedule of administration, and to establish the reversibility of observed toxic effects. In the case of taxol, the toxicity was most evident in tissues with high cell turnover, such as bone marrow and the gastrointestinal tract, but all these toxicities were reversible.

Clinical development - On completion of preclinical studies and favorable review by the NCI staff, all the necessary data are collated and submitted to the FDA as an Investigational New Drug Application (INDA). Once the U.S. Food and Drug Administration (FDA) has approved an INDA, the various phases of clinical development may begin. **Phase I** clinical trials are conducted to determine the maximum tolerated dose (MTD) of a drug in humans, and observe the sites and reversibilities of toxic effects. The starting doses administered are generally well below the LD_{10} determined for mice, and doses are gradually escalated until toxic effects are observed. Phase I trial patients are most often terminally ill and, as with all trials, they are entered on a voluntary basis. Due to the advanced state of disease, meaningful responses of the patients to drug treatment in Phase I trials may not occur, though instances of partial and occasionally complete remissions of various cancers have been noted with certain drugs, such as taxol (Arbuck and Blaylock 1995).

Once the MTD has been determined, and NCI staff are satisfied that no insurmountable problems exist with toxicities, the drug advances to **Phase II** clinical trials. The trials are generally conducted to test the efficacy of the drug against a range of different cancer disease-types. In Phase II trials, doses at, or close to, the MTD level are administered, and patients are evaluated for meaningful response to the drug treatment. Additional confirmatory Phase II trials may be conducted against those disease-types showing meaningful responses. **Phase III** clinical trials are conducted against those disease-types responding to the new drug treatment, and the efficacy of the drug is compared with that of the best chemotherapeutic agents currently available for those disease-types. 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.

In Phase I trials of taxol, the major toxicity observed was neutropenia, with nausea and alopecia (hair-loss) also evident, but all of these were reversible on

cessation of drug treatment. As mentioned earlier, severe allergic reactions were observed with some patients, and these were determined to be due to the large amount of the emulsifying agent, Cremophore EL, in the formulation vehicle. These adverse reactions were overcome by administering the drug as a slow infusion, and pretreatment of patients with anti-allergic regimens. In Phase II trials, significant responses were observed in the treatment of patients with ovarian and breast cancers, while promising results have been obtained against other cancers, such as lung and head-and-neck cancers. Trials are continuing against other cancer disease-types, and Phase III studies are being conducted to define the activity of taxol in combination with cisplatin, and other drugs active in ovarian cancer.

Once sufficient evidence has been accumulated indicating that the new drug is effective for a particular disease type, all the necessary information is assembled and filed as a New Drug Application (NDA) with the FDA. The NDA generally will apply only to the particular responsive disease-type, and approval by the FDA usually only permits marketing of the drug for use in the treatment of that disease-type. Taxol is currently approved for the treatment of breast and ovarian cancers.

Analogue development - In some instances, the active natural product originally isolated from the plant or other organism might not prove to be suitable for development as the final clinical drug. Reasons for not selecting the natural product for clinical development could include insurmountable formulation problems, unacceptable toxicity, or metabolism *in vivo* to inactive metabolites. In such cases, medicinal chemists will attempt to overcome the problem(s) by the synthesis of active derivatives and analogues, and the determination of structure-activity relationships (SAR). A notable example of this strategy is the semisynthesis of the anticancer agent, etoposide (Fig. 3.2) from epipodophyllotoxin, an epimer of podophyllotoxin (Jardine 1979). Podophyllotoxin, isolated from *Podophyllum peltatum* or *P. emodii*, proved to be unacceptably toxic in early clinical trials; extensive research led to the development of etoposide. Etoposide shows clinical activity against small-cell lung and testicular cancers, as well as lymphomas and leukemias (O'Dwyer *et al.* 1985). A more recent example is that of camptothecin (Fig. 3.4), isolated from *Camptotheca acuminata* (Suffness and Cordell 1985b). Clinical trials of a soluble salt of camptothecin in the U.S. in the 1970s were discontinued due to observation of severe toxic effects, but recently several new camptothecin derivatives have entered clinical development in the U.S., Europe and Japan. These derivatives, which are showing activity against leukemias, lymphomas, ovarian cancer, and various forms of lung cancer, are prepared by semi-synthesis from natural camptothecin obtained from Chinese and Indian sources (Wall and Wani 1993).

Costs and timespans of drug discovery and development - The preceding discussion illustrates the complexities of the drug discovery and development process, with particular reference to anticancer drugs. Though the percentage of natural product extracts showing preliminary activity in an *in vitro* screen might

vary from less than 1% to 5%, the number of potentially valuable "leads" from plant and animal sources is more likely to be one in 5,000 to 10,000. Such "leads" will undergo extensive research and development, and probably less than 50% of those will advance to commercial drug status. Considering the NCI anticancer screening program from 1960 to 1982, of the 114,000 plant extracts screened, only taxol has advanced to final FDA approval, while camptothecin has yielded several semisynthetic derivatives which show clinical promise and might advance to commercial status. Homoharringtonine is showing efficacy against certain leukemias, and eventually might be developed as a second-line anticancer agent. Meanwhile, considerable resources were devoted to the development to clinical trials of nine other agents, including bruceantin, camptothecin, indicine N-oxide, maytansine, and thalicarpine, only to have trials terminated due to lack of efficacy or unacceptable toxicity; many other agents were entered into preclinical development but dropped for various reasons (for examples see Suffness and Cordell 1985a). The chances of developing an effective commercial anticancer drug, such as taxol, therefore, are of the order of one in 40,000 to 50,000, based on number of plant extracts screened. The timespan for development can vary considerably, and can be from 10-20 years for anticancer drugs. Research on the isolation of taxol started in the mid-1960s and its structure was first published in 1971 (Wani *et al.* 1971). Its development was delayed for various reasons (Cragg *et al.* 1993b), but, once efficacy against refractory ovarian cancer was observed in late 1988 (Arbuck and Blaylock 1995), advancement to final FDA approval was relatively rapid.

In the light of the time and resources which are required for development of a commercial drug, and the resources devoted to eventual failed candidates, it is not surprising that cost estimates for drug discovery and development exceed U.S. $230 million (DiMasi *et al.* 1991).

Discovery and development of Michellamine B

Michellamine B (Fig. 3.7) was isolated as the main active 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* (Thomas and Gereau 1993). 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 (Boyd *et al.* 1994). 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 current collections of these leaves should provide sufficient

biomass for the isolation of enough drug for completion of preclinical development and possible preliminary clinical evaluation. It is clear, however, that extensive collections of fresh leaves could pose a possible threat to the wild

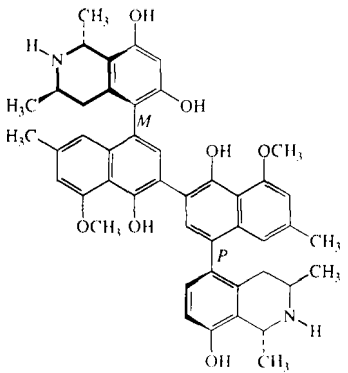


Fig. 3.7. Michellamine B.

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. An extensive botanical survey has been undertaken, and the range and distribution of the species has been 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 has been established to hold and maintain the *A. korupensis* collection at the Korup Park headquarters in Mundemba. The cultivation project has been coordinated by the Center for New Crops and Plant Products of Purdue University, working in close collaboration with the University of Yaounde I, the World Wide Fund for Nature Korup Project, Missouri Botanical Garden, Oregon State University and the NCI contractor, SAIC. In keeping with the NCI policies of collaboration with source countries, all the cultivation studies are being performed in Cameroon, and involve the local population, particularly those in the regions adjacent to the Korup National Park. In performing this project the cooperation and support of the scientific community and the Government of the Republic of Cameroon has been indispensable, and is greatly appreciated by the NCI and its collaborating organizations.

Based on the observed activity, the NCI has committed michellamine B to INDA-directed preclinical development. Unlike many natural products, formulation presents no problem since the drug is readily water-soluble as its diacetate salt. Continuous infusion studies in dogs indicate that *in vivo* effective anti-HIV concentrations can be achieved at nontoxic dose levels. However,

despite these observations and the *in vitro* activity against an impressive range of HIV-1 and HIV-2 strains, there are some serious disadvantages which could preclude advancement of michellamine B to clinical trials. The difference between the toxic dose level and the anticipated level required for effective antiviral activity is small, indicative of a very narrow therapeutic index. In view of some of the toxicities observed in the toxicology studies this narrow therapeutic index is a concern to clinicians considering the drug as a candidate for preliminary clinical trials. In addition, administration by continuous infusion over a period of several weeks is a decided disadvantage compared to oral administration, but, unfortunately, michellamine B is not orally bioavailable.

Even if michellamine B does show some activity in a preliminary clinical trial (assuming it advances that far), it is clear that extensive research will be necessary to determine if 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 Professor Bringmann and his group reported in these Proceedings could provide a satisfactory solution (see chapter 1). The isolation of the novel antimalarial compounds, the korupensamines, from *A. korupensis*, provides another class of potential medicinal agents from this plant (Hallock *et al.* 1994). 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. The development of the michellamines and/or the korupensamines as effective medicinal agents will require true international collaboration between all parties if Cameroon, as the original source country, is to derive optimal benefits from these significant discoveries.

Potential for international collaboration

The discovery and development of michellamine B illustrates the potential for international collaboration resulting from the contract collection programs supported by the NCI. Further examples of productive collaborations are the development of the calanolides, isolated from the Sarawak (Malaysia) plants, *Calophyllum lanigerum* and *C. teysmanii* (Kashman *et al.* 1992), and conocurvone, isolated from the Western Australian *Conospermum* species (Decosterd *et al.* 1993). In these instances, NCI is collaborating respectively with the Sarawak State Government, and the Western Australian Conservation and Land Management agency (CALM) and the Australian pharmaceutical company, AMRAD.

In addition to the contract acquisition programs, direct collaborations have been established between the NCI and research organizations in countries not covered by the present collection contracts, or organizations studying organisms not included in the NCI program. Medicinal plants from Yunnan Province of the Peoples' Republic of China, Korea, India and Russia are being studied in collaboration with the Kunming Institute of Botany, the Korean Research Institute

of Chemical Technology in Seoul, the Central Drug Research Institute in Lucknow, and the Cancer Research Center in Moscow, respectively. Collaborations have also been established with Instituto Nacional de Biodiversidad (INBio) in Costa Rica, the South American Organization for Anticancer Drug Development in Brazil, the Institute of Chemistry at the National University of Mexico, the HEJ Research Institute in Karachi, Pakistan, and the Zimbabwe National Traditional Healers Association (ZINATHA) and the University of Zimbabwe. In establishing these collaborations, NCI undertakes to abide by the same policies of collaboration and compensation as apply to source countries participating in the contract collection programs.

The terms of collaboration are generally stated in a Memorandum of Understanding (MOU) signed by the organization and the NCI. The terms, based on the policies of the NCI Letter of Collection, are summarized in a schematic diagram (Fig. 3.8) and are presented in detail in thé "generic" MOU (Appendix 3.1).

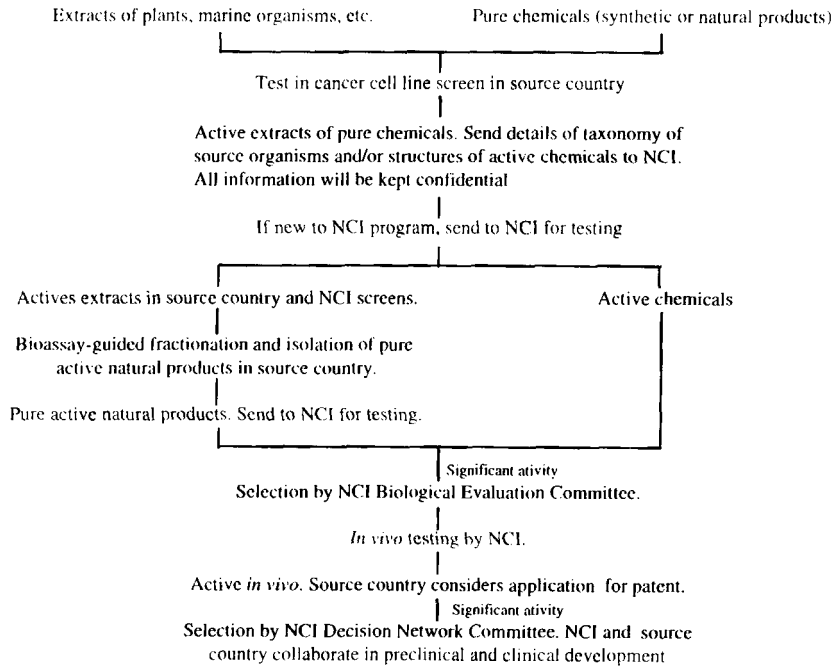


Fig. 3.8. Policy of the NCI in term of possible collaboration between NCI and other organization.

When suitable screening facilities are available in-country, the organization tests pure compounds and/or extracts and provides a list of the identified active materials to the NCI in order that the NCI databases may be checked for earlier

submissions. This is a critical requirement, since the NCI has received thousands of compounds and raw materials (plants, marine organisms, etc.) from suppliers worldwide, either on a voluntary basis or through contract mechanisms. It is most important to avoid duplications, since the NCI has legal obligations to suppliers and source countries to protect their rights and intellectual property which could be crucial factors in determining terms of collaboration and compensation should a commercial product be developed. In the case of raw materials, or extracts thereof, if the prior submissions were inactive in the NCI screens, consideration may be given to accepting additional samples since the chemical constituents and bioactivity of plants and other organisms are known to vary depending on the location and time of collection. Those materials new to the NCI program (or extract samples of duplicate organisms originally found to be inactive) are submitted to the NCI *in vitro* screens, and the results provided exclusively to the relevant suppliers. In the case of active pure compounds, additional samples may be requested for *in vivo* testing. Significant *in vivo* activity, and selection by the NCI Decision Network Committee for preclinical development, would merit serious consideration of patent application by the supplier organization to cover the active compound and related derivatives and analogues thereof. In the case of active extracts, bioassay-guided fractionation and isolation of the active constituent(s) may be pursued independently by the organization using in-country screens, or the organization may designate a scientist to visit the NCI facilities in Frederick or equivalent facilities at an approved U.S. institution (*e.g.* a university) to participate in joint isolation studies. The development of any isolated active constituent then follows the same path as described for pure compounds above. If the isolation of an active constituent was performed independently by the supplier organization, patent application would be pursued by the organization at its own cost, and patent rights would belong exclusively to the organization. If, however, the isolation studies were performed jointly by the supplier organization and the NCI or an approved U.S. organization, patent application would be pursued jointly by the collaborating parties with shared costs, and patent rights would be shared by these parties. All information generated and exchanged during the collaboration is considered confidential, with presentation and publication being subject to mutual agreement of the parties involved.

Once an active compound is selected by the NCI Decision Network Committee for preclinical and clinical development, it enters into drug development. The Decision Network Process divides the drug development process into stages designated as DNIIA, DNIIB and DNIII (Fig. 3.9). The NCI Developmental Therapeutics Program (DTP/NCI) collaborates closely with the supplier organization in this process, and the division of tasks depends on the capabilities of the supplier organization. Most pharmaceutical companies can support all aspects of the drug development process, but other suppliers organizations generally do not have the expertise and resources to accomplish most phases of drug development. In such instances, the supplier organization may provide additional quantities of the active agent, while the DTP/NCI will devote its resources to the remaining phases. DTP/NCI can also provide support to the

supplier organization to produce additional quantities of the active agent, if necessary. Should the organization be unable to provide the additional quantities in a reasonable period of time, the DTP/NCI will require authorization to prepare them utilizing procedures provided by the organization.

As mentioned earlier, drug development is an extremely costly process, and, once the DTP/NCI has committed itself to the development of a drug, it may expend considerable resources amounting to millions of dollars. Therefore, should a supplier organization decide to cease collaboration at any stage, the DTP/NCI reserves the right to proceed with the development utilizing whatever means it considers appropriate. Even in such an unlikely event, the supplier organization and the corresponding source country still stand to benefit should a commercial product eventually result.

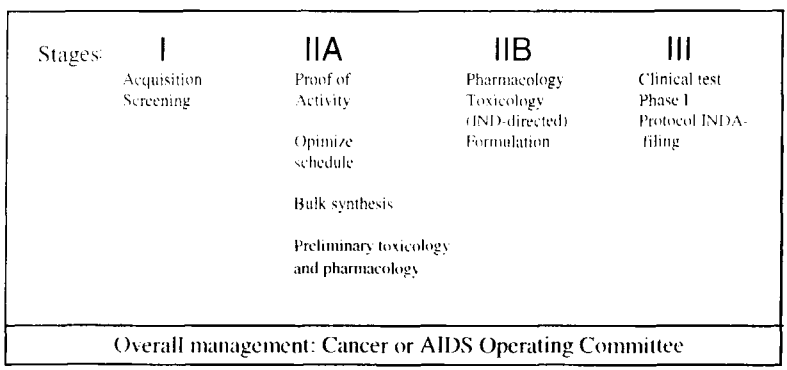


Fig. 3.9. NCI Decision Network Process.

The above discussion primarily applies to research organizations in genetically-rich source countries wishing to collaborate in the investigation of their natural resources. In the case of organizations only wishing to have pure compounds tested, 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 at universities and pure research organizations wishing to submit pure compounds for testing would probably consider entering into this agreement as opposed to the MOU discussed earlier.

Routine/non-proprietary screening and testing versus non-routine/proprietary screening and testing (see Appendix 3.2)

This issue, referred to above, designates those NCI operations which could result in intellectual contributions in the development of a compound by NCI scientists,

and which may rise to the level of inventorship as determined under United States patent law. Routine/non-proprietary screening and testing (Step 1) generally refers to the standard NCI *in vitro* and *in vivo* anticancer and anti-HIV screens, and preliminary formulation and toxicology studies necessary to perform the animal *in vivo* screens. Non-routine/proprietary screening and testing (Step 2) encompasses more advanced formulation, pharmacological and toxicological studies aimed at determining optimal parameters for clinical trials, as well as analogue development and mechanism of action studies. These latter operations require significant research involving intellectual input which could result in sole NCI scientist inventorship. In signing either an MOU or a screening agreement, NCI agrees that it will not proceed with Stage II operations without the prior written consent of the supplier organization. In addition, the supplier organization may designate which of the Stage II operations it wishes to delete from the scope of the MOU or screening agreement prior to execution, and DTP/NCI could consider amending the agreement to incorporate them at a later stage. If no limitation in the scope of testing is requested by the supplier organization, it is assumed that DTP/NCI may proceed directly from Stage I to Stage II testing. Should a compound show promising anticancer or anti-AIDS activity through Stage I and designated Stage II testing, the NCI will propose the establishment of a more formal collaboration.

Conclusions

The NCI has the full capability to advance compounds showing promising preliminary anticancer or anti-AIDS activity through all the phases of preclinical and clinical development. The NCI welcomes the opportunity to collaborate with organizations in the discovery and/or development of anticancer and anti-AIDS agents, and has formulated agreements (Memorandum of Understanding or Screening Agreement) outlining terms of collaboration covering issues of confidentiality, inventorship and patent rights, and compensation should a commercial product be developed. The NCI, as a U.S. taxpayer supported, non-profit institution, is firmly committed to protecting the rights of suppliers and Source Countries during all phases of drug discovery and development.

Acknowledgments

NCI gratefully acknowledges the collaboration and support of the many individuals and organizations worldwide which make these programs possible. From the collection of organisms in over 25 countries to the clinical trials of new drugs, and the studies of occurrence and prevention, this is truly an international effort in the fight against the scourges of AIDS and cancer. NCI recognizes the indispensable contributions being made through the provision of valuable natural resources, expertise, knowledge, and skills; through policies of collaboration and

compensation, as stated in the Letter of Collection, NCI wishes to assure participating countries of its commitment to working with them in a fair and equitable manner.

References

Arbuck, S.G. and Blaylock B.A. (1995). Taxol: Clinical results and issues in development. In *Taxol®: Science and Applications* (ed. M. Suffness), pp. 379-415. CRC Press, Inc., Boca Raton, Florida, U.S.A.

Boyd, M.R. (1988). Strategies for the identification of new agents for the treatment of AIDS: A national program to facilitate the discovery and preclinical development of new drug candidates for clinical evaluation. In *AIDS: Etiology, Diagnosis, Treatment and Prevention* (eds V.T. Devita, S. Hellman and S.A. Rosenberg), pp. 305-319. J.B. Lippincott, Philadelphia.

Boyd, M.R. (1989). Status of the NCI preclinical antitumor drug discovery screen. In *Principles and Practice of Oncology Updates, Vol 3, No. 10 Prevention* (eds V.T. Devita, S. Hellman and S.A. Rosenberg), pp. 1-12. J.B. Lippincott, Philadelphia.

Boyd, M.R. (1993). The future of new drug development. In *Current Therapy in Oncology, Section I. Introduction to Cancer Therapy* (ed. J. Niederhuber), pp. 11-22. B.C. Decker, Inc., Philadelphia.

Boyd, M.R., Hallock, Y.F., Cardellina II, J.H., Manfredi, K.P., Blunt, J.W., McMahon, J.B., Buckheit Jr, R.W., Bringmann, G., Schaffer, M, Cragg, G.M., Thomas, D.W., and Jato, J.G. (1994). Anti-HIV michellamines from *Ancistrocladus korupensis*. *Journal of Medicinal Chemistry* **37**, 1740-1745.

Cassady, J.M. and Douros, J.D.(eds.). (1979). *Anticancer agents based on natural product models*. Academic Press, New York.

Cragg, G.M. and Suffness, M. (1988). Metabolism of plant-derived anticancer agents. In *Pharmacological Therapy, 37* (G. Powis (ed.), pp. 425-461. Pergamon Press, Oxford.

Cragg, G.M., Boyd, M.R., Cardellina II, J.H., Grever, M.R., Schepartz, S.A., Snader, K.M., and Suffness, M. (1993a). Role of plants in the National Cancer Intitute Drug Discovery and Development Program. In *Human Medicinal Agents from Plants. ACS Symposium Series* 534 (eds. A.D. Kinghorn and M.F. Balandrin), pp. 80-95. American Chemical Society, Washington, D.C.

Cragg, G.M., Schepartz, S.A., Suffness M., and Grever, M.R. (1993b). The taxol supply crisis. New NCI policies for handling the large-scale production of novel natural product anticancer and anti-HIV agents. *Journal of Natural Products* **56**, 1657-1668.

Davignon, J.P., and Cradock, J.C. (1987). *The Formulation of Anticancer Drugs*, pp. 212-227. McGraw-Hill, Inc., New York.

Décosterd, L.A., Parsons, I.C., Gustafson, K.R., Cardellina II, J.H., McMahon, J.B., Cragg, G.M., Murata, Y., Pannel, L.K., Steiner, J.R., Clardy, J., and Boyd, M.R. (1993). Structure, absolute sterochemistry, and synthesis of conocurvone, a potent, novel HIV-inhibitory naphthoquinone trimer from a *Conospermum* species. *Journal of the American Chemical Society* **115**, 6673-6679

DiMasi, J.A., Hansen, R.W., Grabowski, H.G., and Lasagna, L. (1991). Cost of innovation in the pharmaceutical industry. *Journal of Health Economics* **10**, 107-142.

Jardine, I. (1979). Podophyllotoxins. In *Anticancer Agents Based on Natural Product Models* (eds. J.M. Cassady and J.D. Douros), pp. 319-351. Academic Press, New York.

Kashman, Y., Gustafson, K.R., Fuller, R.W., Cardellina, II, J.H., McMahon, J.B., Currens, M.J., Buckheit, R.W., Hughes, S.H., Cragg G.M., and Boyd, M.R. (1992). The Calanolides, a novel HIV-inhibitory class of coumarin derivatives from the rainforest tree, *Calophyllum lanigerum*. *Journal of Medicinal Chemistry* **35**, 2735-2743.

O'Dwyer, P., Leyland-Jones, B., Alonso, M.T., Marsoni, S., and Wittes, R.E. (1985). Etoposide (VP-16-213). Current status of an active anticancer drug. *New England Journal of Medicine* **312**, 692-700.

Philip, P.A., Rea, D., Thavasu, P., Carmichael, J., Stuart, N.S.A., Rockett, H., Talbot, D.C., Ganesan, T., Pettit, G.R., Balkwill, F., and Harris, A.L. (1993). Phase I study of Bryostatin 1: assessment of interleukin 6 and tumor necrosis factor a induction *in vivo*. *Journal of the National Cancer Institute* **85**, 1812-1818.

Suffness, M. and Cordell, G.A. (1985a). Academic Press, New York.

Suffness, M. and Cordell, G.A. (1985b). Camptothecin. In *The Alkaloids, Vol. 25* (ed. A. Brossi), pp. 73-89. Academic Press, New York.

Thomas, D.W. and Gereau, R.E. (1993). *Ancistrocladus korupensis* (Ancistrocladaceae): A new species of liana from Cameroon. *Novon* **3**, 494-498.

Wall, M.E. and Wani, M. (1993). Camptothecin and analogues: Synthesis, biological *in vitro* and *in vivo* activities, and clinical possibilities. In *Human Medicinal Agents from Plants. ACS Symposium Series* 534 (ed. A.D. Kinghorn, and M.F. Balandrin), p. 149. American Chemical Society, Washington, D.C.

Wani, M.C., Taylor, H.L., and Wall, M.E. (1971). Plant Antitumor Agents. VI. The isolation and structure of taxol, a novel antileukemic and antitumor agent from *Taxus brevifolia*. *Journal of the American Chemical Society* **93**, 2325-2327.

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