ETHNOPHARMACOLOGY *and the* SEARCH FOR NEW DRUGS

by Paul Alan Cox

PLANT NATURAL PRODUCTS AND NEW DRUGS

Natural products derived from higher plants may contribute to the search for new drugs in three different ways: (1) by acting as new drugs that can be used in an unmodified state, e.g. vincristine from CATHARANTHUS ROSEUS *(Apocynaceae); (2) by providing chemical "building blocks" used to synthesize more complex compounds, e.g. diosgenin from* DIOSCOREA FLORIBUNDA *(Dioscoreaceae) for the synthesis of oral contraceptives; and (3) by indicating new modes of pharmacological action that allow complete synthesis of novel analogues, e.g. synthetic analogues of reserpine from* RAUWOLFIA SERPENTINA *(Apocynaceae). Natural plant products are also used directly in the "natural" pharmaceutical industry that is growing rapidly in*

Europe and North America, as well as in traditional herbal medicine programs being incorporated into the primary health care systems of Mexico, the People's Republic of China, Nigeria, and other developing countries.

The importance of plant-derived pharmaceuticals can be deduced from their prominence in the market place: Farnsworth's 1984 analysis of the National Prescription Audit of 1976 revealed that 25% of all prescriptions issued in the United States and Canada contained an active component derived or originally isolated from higher plants. The need to examine the plant kingdom for new pharmaceuticals is particularly urgent in light of the rapid deforestation of the tropics and the concurrent loss of biodiversity throughout the world.

Why do plants produce bioactive compounds? With the exception of cultivated drug plants such as kava, whose mixture of kavalactones (structurally similar to sesquiterpenical lactones) has been produced through artificial selection by many generations of Pacific islanders (Lebot 1990), the pharmacological activity of most plants must be regarded as fortuitous. Bioactive molecules occur in plants primarily as secondary metabolites and/or chemical defenses against predation, herbivores, fungal attack, microbial invasion, and viral infection.

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Random and Targeted Screens of Floras for Bioactive Molecules

Modern searches for plants with bioactive molecules are typically based on some sort of bioassay that will detect specific bioactivity within a crude plant extract. Should activity be found, isolation, purification, and structural determination of the bioactive molecule are then pursued. Given limited finances for such searches, it is clear that not every one of the 250,000 different species of flowering plants and thousands of conifers, ferns, and bryophyte species can be studied for useful bioactivity. How then to decide which plant species should be selected for testing?

Two different types of plant selection programs have traditionally been attempted in the search for bioactive molecules from plants. The first general type of program I characterize as "random," while the second general program can be termed "targeted."

In random plant selection programs, plants are collected and screened without regard to their taxonomic affinities, ethnobotanical context, or other intrinsic qualities. Many large scale screening programs of the 1960s, funded by both the private sector and governmental agencies, initiated essentially random screens of floras. Collectors were dispatched to various parts of the world and instructed to collect as many different types of plants as possible. Although some random screens were designed to ensure adequate botanical rigor, stories still circulate among the botanical fraternity of ill-trained collectors, usually chemists, hastily scribbling field notes directly on the leaves of plants, grinding up voucher specimens, and making radical mis-identifications of plants collected. While most of these stories were undoubtedly apocryphal, many screening programs obviously lacked botanical rigor. Yet even rigorous random searches had an extremely low success rate.

Perhaps the lack of interest of the pharmaceutical industry in natural plant products during the 1970s and early 1980s stemmed in part from the failure of random searches. An exception to the rather dismal success rate is Taxol, an antitumor compound developed by the National Cancer Institute from *Taxus brevifolia* (Taxaceae), which is found in British Columbia.

The alternative to random plant surveys is targeted surveys. These can be of several types. In phylogenetic surveys, the close relatives of plants known to produce useful compounds are collected and analyzed for either novel homologues or increased concentrations of the compounds of interest. For example, vincristine and vinblastine occur in very low concentrations in *Catharanthus roseus*; analysis of the close relatives of the taxon may reveal the same or similar vinca alkaloids in higher concentrations. In ecological surveys, plants living in particular habitats or possessing certain types of growth habits are chosen for study. For example, I am very interested in plants occurring in areas where slug and snail predation of seedlings is high—my hope is to find an inexpensive molluscicidal compound that could aid in the fight against schistosomiasis in central Africa. Finally, in ethnopharmacological surveys, plants used by indigenous peoples in traditional medicine are chosen for study. I will address the remainder of my remarks to this final category.

ETHNOPHARMACOLOGICAL SCREENS AND THE SEARCH FOR BIOACTIVE COMPOUNDS

It is of interest that most of the drugs derived or originally isolated from higher plants were discovered in an ethnobotanical context. For example, reserpine, derived from *Rauwolfia*, was discovered by analyzing Indian Aryuvedic remedies. This is not too startling since many natural products from European plants, such as scopolamine or digitalis, were originally discovered because of their use in crude extracts in folk medicine. Yet such discoveries have continued well into the 20th century, perhaps the most significant being the discovery of vinca alkaloids vincristine and vinblastine from *C. roseus*. This was the 40th of 200 plant species examined by Eli Lilly and Co. from a collection of medicinal plants used by the indigenous people of Madagascar (Tyler 1986).

The history of drug discovery and development seems to confirm that ethnobotanical screens of floras have a far higher chance of success than random screens. But not all cultures are equally likely to use plants with significant pharmacological activity. Three features characterize indigenous cultures that probably do use such plants.

First, the culture must possess a conservative ethnomedical tradition with established mechanisms for the accurate transmission of ethnopharmacological knowledge. Cultures that have been using the same medicinal plants for many generations are more likely to have identified plants with useful bioactivity.

Second, the culture should reside in an area with a diverse flora. The high plant diversity should increase the probability of discovering plants with useful bioactivity. Using this rule, the Alyut tribes of northern Alaska are unlikely to have identified as many bioactive plants as the indigenous cultures of the Amazon basin, all else being equal.

Third, there should be a continuity of residence in the area over many generations. This permits more time for cultural exploration of local floral resources. Under this rule, the use of indigenous plants by the European settlers of Australia would be of less pharmacological interest than that by aboriginal peoples who have lived there for 19,000 years.

Ethnopharmacological data derived from cultures possessing all three of these characteristics are, in a sense, analogous to human bioassay data, particularly if people have been dosing themselves with the same plants for many generations. In such situations, preparations with low efficacy or acute toxicity are likely to have been discarded over the years. Thus, I argue that the greatest probability of discovering useful bioactive compounds from plants lies in careful examination of pharmacopoeias of cultures possessing the three characteristics I have outlined.

In unmonetized cultures, financial interests can be translated into conservation efforts to preserve and protect rain forest and other habitats valued by the healers.

EMPIRICAL VINDICATION OF THE EFFICACY OF ETHNOPHARMACOLOGICAL SCREENS

Is there any non-anecdotal evidence that indigenous pharmacopoeias are likely to contain plants with bioactive molecules? A recent survey of the Samoan ethnopharmacopoeia revealed that 86 percent of the plant species used in Samoan traditional herbal medicine show pharmacological activity in broad *in vitro* and *in vivo* screens (Cox et al. 1989); 74 different species were tested for activity in a Hippocratic screen and a guinea pig ileum test. Further studies of Samoan medicinal plants have resulted in the isolation of a triterpene from *Alphitonia zizyphoides* (Rhamnaceae), which inhibits the formation of prostaglandin *in vitro* and EPP-induced edema in the rat ear (P. Perera et al., unpublished) and antiviral compounds from *Homalanthus acuminatus* (Euphorbiaceae) in Samoa (K. Gustaffson et al., unpublished). Similar results from studies of other ethnopharmacopoeias are being reported.

Unfortunately, though, few research teams have the necessary skills to initiate an ethnopharmacological screen. At the minimum such a team should have a core of two professionals: an ethnobotanist and a pharmacognosist. Pharmacology and botany have been historically disparate disciplines with differing approaches, and ethnobotany and pharmacognosy are the two disciplines most likely to bridge the gap between them in the search for novel pharmacological substances. Careful ethnopharmacological investigations by trained ethnobotanists are crucial not only in collecting the plants that will be later analyzed, but also in documenting the methods or formulation, administration, and general cultural context of the plant use. Since the subsequent tasks of pharmacological screening and natural product chemistry are per-

haps better understood by the pharmaceutical community, I will briefly describe the tasks of ethnobotanists in the search for new drugs.

ETHNOBOTANISTS AND THE SEARCH FOR BIOACTIVE MOLECULES

Ethnobotanists are professionals with both botanical and anthropological training. Ideally they should learn the language and customs of the people they work with, because it is their task to serve as bridges between different cultures, as facilitators of communication between the indigenous healers and the other scientists in the research team.

In the field, ethnobotanists are responsible for identifying the healers in an indigenous culture and securing their participation in the research program. Such cooperation is usually granted, not only because of the language skills and cultural abilities of the ethnobotanist, but also because of the common knowledge and interest in plants shared by ethnobotanist and healer. Through extensive interviews, participant observation methodologies, and in some cases actual apprenticeships, ethnobotanists attempt to learn the ways of the healers and make their knowledge accessible to other Western scientists. This is rarely an easy task, since medicinal plants are only a single component of indigenous ethnomedicines.

THE THREE COMPONENTS OF ETHNOMEDICINE

All indigenous medical traditions share three components: a cosmological foundation, a repertoire of medicinal plants or other pharmacologically active substances, and a health care delivery system. An appreciation of all three components is necessary for rigorous ethnobotanical studies. For example, indigenous disease categories rarely can be mapped one-to-one on to Western disease categories; hence, understanding of the cosmological foundations of indigenous disease taxonomies and aetiologies is necessary to understand indigenous disease terms. Similarly, indigenous health care delivery systems are usually very different from Western ones; fruitful cooperation with indigenous healers requires respect for local systems.

A rigorous ethnopharmacological research program requires a high degree of cultural and linguistic sophistication on the part of the ethnobotanist. Rarely can adequate translations be obtained through interpreters, since healers employ specialist concepts and terms that are unknown to most of the members of their culture. As anthropologist Bruce Biggs suggests:

> *If you want to understand fully the ideas of sickness and health that underlie your healer's practices, his categories of disease, and the specialist vocabulary of his profession, you must work in* his *language, not yours, because while you may, eventually, get to understand what he tells you in his language, and translate it into something that can be compared with western ideas on the same topic, there is no way that your informant, perfect though his English may be, can do that for you. His very use of English will mask and obscure your topic of investigation. (Biggs 1985, p 110–111)*

Once rapport is established with the healers and a preliminary understanding of the cosmological foundation of the healing tradition is obtained, collection of the indigenous ethnopharmacopoeia can begin. It is crucial that any collections be documented with copious notes and well-prepared voucher specimens.

The importance of adequate herbarium voucher specimens cannot be overstated: any question or dispute concerning the botanical identity of the species involved can be unequivocally settled by examination of a properly collected voucher specimen. Such a specimen should supply the ethnobotanical information, including informant names, and geographical detail necessary to relocate the plant population should recollection be required (Croom 1983). Because of their scientific value for years to come (indeed, sophisticated bioassays of the future may require only micrograms of plant material), voucher specimens should be deposited and preserved in a well-curated herbarium with duplicate specimens deposited in geographically distant herbaria. The collection number of the voucher specimen should be used to label all subsequent pharmacological fractions and residues, so that any new discovery or question can be immediately referred to the original herbarium sheet.

In addition to preparing voucher specimens, ethnobotanists are also responsible for collecting plant materials for pharmacological testing. Careful attention should be paid to the plant parts collected, because frequently flowers, leaves, shoots, and roots differ significantly in their chemical composition. Dried samples ranging from 0.5 to 2 kg. have been traditionally supplied for testing, but drying, despite its ease, may not always be the best method of preservation. In general, collection and preservation techniques should closely approximate those used by the indigenous healers. Thus in Samoa, where healers prepare water-infusions from fresh plant materials (Cox 1990a,b) I macerate the fresh plants and pack them in 70% ethanol in shatterproof aluminum bottles (Cox et al. 1989). After transportation to the laboratory, the water fraction is freeze-dried while the alcohol fraction is preserved in glass bottles for analysis by gas-phase chromatography.

Should any plants collected show useful bioactivity

during initial screening, documentation should be sufficient to facilitate rapid recollection of material in bulk. Ethnobotanical data on the diseases treated, mode of formulation and methods administration should be comprehensive, to guide subsequent investigations.

OBLIGATIONS OF ETHNOBOTANISTS TO INDIGENOUS PEOPLES

The debt of the research team to the indigenous healers should be remembered during all phases of drug searches based on ethnopharmacological screens. By the time that a drug search has advanced to the phase of organic synthesis, it is easy to forget that the compound in the test tube was originally obtained through the kindness and willingness of traditional healers to share their knowledge. Because of her or his close association with the healers, the ethnobotanist in any research team has a special obligation to represent the interests of the indigenous healers. Certainly the highest obligation of any scientist is to tell the truth, at all times, and in all places. Thus it is crucial that the purpose and nature of the research program be accurately represented to healers before one secures their participation. Second, during the ethnobotanical research every effort should be made to respect and affirm the indigenous culture. As a result, I do not solicit, record, or transmit information that a healer identifies as "secret." I make it clear to the healer at the onset that I intend to publish the information she or he gives me. My experience is that healers are usually delighted to have their knowledge preserved. Wherever possible, I include a native language abstract in the publication, acknowledge the healers by name, and distribute reprints to the healers. Third, I make it clear to my collaborators that the indigenous healers have a financial interest in any marketable compound derived from their knowledge. These interests are written in patent agreements signed by representatives of the institutions and the villages concerned. In unmonetized cultures, financial interests can be translated into conservation efforts to preserve and protect rain forest and other habitats valued by the healers.

CONCLUSION

Although historically many useful bioactive compounds have been identified from indigenous pharmacopoeias, there have been few attempts to screen these carefully for bioactivity. Ethnopharmacological screens are far more likely to discover useful bioactive compounds than are random screens, but they require careful collaboration of ethnobotanists with pharmacognosists, natural product chemists, pharmacologists, toxicologists, and other members of the research team.

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The following article from the September 1992 issue of BYU TODAY *reviews the significance of Paul Cox's research in the battle against the HIV virus and AIDS.*

AIDS KNOWLEDGE MAY GET BOOST FROM SAMOAN RAINFOREST

By Charlene Renberg Winters, STAFF WRITER

Scientists of the National Cancer Institute and BYU are collaborating in studies of a Samoan rainforest plant as a potential candidate for drug development because of its protective action in the test tube against the HIV virus.

Current progress of the NCI-BYU research is detailed in the recent issue of *Journal of Medicinal Chemistry.*

Such rainforest research eventually may help solve a piece of the AIDS puzzle, says BYU ethnobotany professor Paul Cox, who has been working among native Samoan plant healers for eight years.

Cox's collaboration with the NCI Laboratory of Drug Discovery Research and Development and the NCI Natural Products Branch identified a small tree used by native healers to combat possible viral diseases which may also contain fundamental anti-HIV characteristics.

The NCI-BYU team found that prostratin, the active chemical isolated from the medicinal plant *Homolanthus nutans,* deserves further research because it inhibits the growth of the virus associated with AIDS.

In initial tests, scientists dosed normal cells with the virus HIV-1, which killed healthy cells. Yet cells that had an application of prostratin before receiving the virus suffered no damage. The cells were protected against HIV-induced killing even when prostratin was added as late as 24 hours after infection.

"Based on Paul Cox's ethnobotanical studies with Samoan healers, this testing offers a very interesting demonstration of how ethnobotany can be useful in providing leads about health and illness," says NCI's Dr. Gordon Cragg.

As encouraging as the data is, however, additional testing needs to be done. "We know this protects human cells in culture from death by the AIDS virus," Cox says. "But we don't know whether it will work, or is even safe to test in humans.

"AIDS patients, by definition, already have depressed immune systems. It is crucial that nothing be administered to them that might further increase their susceptibility to fungal infection, pneumonia, or other related diseases. We just don't know if prostratin will be safe for testing in humans until extensive further laboratory studies are performed."

The NCI scientists also agree with this note of caution.

"Extensive toxicology checks must also be conducted to ascertain whether it is safe for testing in patients," Cragg says. "Although prostratin has entered a very early stage of pre-clinical drug development, the complete process of drug development can take more than 10 years."

The identification of prostratin as the active element, says Cox, nearly scrapped subsequent research because prostratin is part of organic compounds called phorbol esters which are known tumor-promoters.

"Phorbol esters are used in labs to cause cancer for research purposes," Cox says. "But I thought it was hard to believe prostratin actively promoted tumors when Samoans have used the source plant for 2,000 years." Its uses included being a treatment for yellow fever, another viral disease.

Another research team, from NCI's Laboratory of Cellular Carcinogenesis and Tumor Promotion, tested prostratin specifically for indications of tumor-promoting activity, and not only did it not cause cancer, it also actually had an inhibiting effect. However, NCI scientists are still investigating the possibility that the plant may contain other toxic compounds.

"The botanical knowledge of the Samoan people is tremendous," Cox says. "I learned the process from a 78-year-old woman who received her skills from knowledge passed from generation to generation. I believe much of their work is pharmacologically sophisticated and belies the myth that these healers are uncivilized and primitive."

The discovery of prostratin also demonstrates the need to conserve tropical rainforests worldwide.

"The loggers and bulldozers were already beginning to destroy the forest where we collected prostratin," Cox says. "Had we not been able to raise $85,000 from friends and private individuals necessary to save a small part of the forest, we may never have discovered this promising new compound."

Cox and other scientists have established the Seacology Foundation to preserve Pacific Island rainforests and the indigenous cultures of the islanders.

According to Cox, The National Cancer Institute has applied for patents on the medicinal use of prostratin, which ensures that if this indigenous Samoan plant ever becomes commercially marketable, a significant portion of the proceeds would return to Samoa.