Taxus baccata
lectotype

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Investigator's Report for Scientific Research: Plant Collections from Kauai and Oahu, Hawaii

Principal Investigator: Richard Spjut

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Under a reimbursable agreement, the World Botanical Associates (WBA) supplies the National Cancer Institute (NCI) with 1,000 samples of plants each year from the United States and Territories in search of new anticancer drugs. Samples of selected plant parts, or the entire plant, are collected in quantities to yield 300–500 g dry weight for extraction and testing against 60 different cell lines of cancer. Examples of anticancer drugs discovered from the NCI screening of plant extracts are taxol from stem-bark of *Taxus brevifolia*–originally collected in the state of Washington, and derivatives of camptothecin from stem or twig, leaf and fruit of *Camptotheca acuminata*—a plant native to S China that was collected at a USDA plant introduction station in Chico, California (Perdue et al. 1970).[1]

The WBA follows a systematic approach based on phytogeographical relationships with limitations in regard to a RFP (Request for Proposal) submitted to the NCI (Nov. 2000, Apr. 2001. The limitations consider 5,000 samples already collected within the United States—by the Morton Arboretum (William Hess, PI, 1997–2001). Our analysis—in response to the NCI RFP—revealed most samples were from deciduous forests of the eastern United States. Collections were lacking entirely from Alaska, Washington, and Hawaii. Spjut (1985),[2] in a review of the NCI plant collection program, treated Hawaii as a separate floristic region among 58 floristic regions he recognized in the world. Hawaii is well known for its endemic species—91% (Wagner, Herbst and Sohmer, *Manual of the Flowering Plants of* Hawai'i, revised ed., 1999, 2 Vols.).

Because the Hawaiian flora is largely endemic and because few previous collections have been screened from Hawaii (since 1986), any indigenous plant there is a candidate for collection except for sensitive species, which includes those classified as threatened or endangered by the US Fish and Wildlife.

Vouchers are prepared for all species sampled. The PI (Richard Spjut) identifies plants in the field using manuals on the local flora before sampling. Identifications of vouchers are reviewed by Roger Sanders except Asteraceae by Guy Nesom, Botanical Research Institute of Texas

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[1] R. E. Perdue Jr. , R. L. Smith, M. E. Wall, J. Hartwell & B. Abbott, *Camptotheca acuminata Decaisne [Nyssaceae] Source of Camptothecin, an Antileukemia Alkaloid*, USDA Tech. Bull. 1415, 1970.
[2] R. W. Spjut, *Limitations of a Random Screen: Search for New Anticancer Drugs in Higher Plants,* Economic Botany 39: 266-288, 1985.

(BRIT). An additional set of vouchers is then sent to the US National Herbarium (US), and to other institutions as may be required by permits.

Summary of Accomplishments

The WBA was not permitted to collect in the public reserves or parks with the more abundant and accessible native species. Notwithstanding, 354 general samples were collected from 79 Hawaiian species; 42 species on Kauai and 37 species on Oahu, of which 61% are indigenous. These are listed in Appendix I. The 61% native species is considerably greater than what may have been previously screened by the NCI from Hawaii—prior to 1986, ~14% for those reported active from Hawaii, Appendix II. This suggests that many species we (WBA) collected are new not only to the present screen, but also new since the program's inception. The chance discovery of novel compounds was further increased by the collection of plant parts most likely to show bio-chemical activity—such as root (or separate samples of root-bark and root-wood), stem-bark, and inflorescence parts (if) in flower (fl) or fruit (fr), 42% of these kinds of samples are represented, compared to ~15% for previous collections from Hawaii—obtained during the 1970's.

Detail

History of the NCI Natural Products Screen

The NCI has been screening plant extracts since 1956.[3] A primary impetus for this program is two plants that were recognized to have potentially useful compounds for treating cancer, the vinca alkaloids in the Madagascar periwinkle (C*atharanthus roseus*), and the lignan, podophyllotoxin, in may-apple (*Podophyllum peltatum*). Compounds from both have led to drugs currently used for treating cancers, vincristine and vinblastine (vinca alkaloids) for leukemia, Hodgkin's lymphoma, breast and colorectal cancers, and derivatives of podophyllotoxin (e.g., etoposide) for bronchial, lymphoma and testicular cancers.

Camptothecin was the first major discovery by the NCI in a random screening of plant extracts from which the isolation and characterization of camptothecin was carried out by Monroe Wall's group at Research Triangle Institute (Wani & Wall, J. Org. Chem. 34: 1364, 1969). Between 1956 and 1960, the NCI screened ~1,500 plant extracts of which ~1,000 were obtained by J. Hartwell (at NCI) from the USDA Plant Steroidal Investigations, headed by Monroe Wall under Chief Willaman, Eastern Utilization Research and Development Division in Wyndmoor PA (Shephartz 1976;[4] Perdue et al. 1970, loc. cit.). The USDA, who had been screening plant extracts for sources of cortisone since 1950, no longer needed the extracts—from samples collected at their plant introduction stations in California, Florida, Georgia and Maryland. Among these was a leaf extract of *Camptotheca acuminata* (Nyssaceae) from Chico, California. It showed preliminary activity in two tumors, but more material was needed for confirmation. In July 1960, the NCI began a collaborative agreement with the USDA Agricultural Research Service (ARS), New Crops Research Branch, in Beltsville MD, and also with the University of Arizona, for collection and extraction of plant samples, while Monroe

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[3] Abbott, *Bioassay of Plant Extracts for Anticancer Activity*, Cancer Treat. Rep. 60: 1007–1010, 1976.
[4] Shephartz, *History of the National Cancer Institute and the Plant Screening Program*; Cancer Treat. Rep. 60: 975–977, 1976.

Wall relocated to the Research Triangle Institute in Durham NC where he later obtained a recollection of *C. acuminata*, among other species, under a contract with the NCI to isolate active agents that commenced in 1961. Additional samples of stem or twig, leaf and fruit of *C. acuminata* were obtained by the ARS in 1961 from Chico; however, an extract from a new leaf sample failed to confirm, although extracts from stem and fruit did confirm (Perdue et al. 1970, loc. cit; CPAM 1978).[5] Derivatives of camptothecin (topetecan and irinotecan)—that have since been discovered—have led to FDA approved drugs for use in treating lung, ovarian and colorectal cancers.

Between 1960 until 1982, the NCI screened ~120,000 extracts from ~35,000 plant species[6] that were supplied by the ARS and other institutions such as the University of Hawaii, Commonwealth Scientific and Industrial Research Organization (Australia), Central Drug Research Institute (India), National Defense Medical Center (Taiwan), University of Arizona, University of Costa Rica, University of Concepcion (Chile), and University of Brazil (Rio De Janeiro). Richard Marin (World Botanical Associates), who collected with Bruce Halstead, supplied specifically ethnobotanical samples to the NCI through the University of Loma Linda (see also worldbotanical.com for those collected in Africa by Halstead), and later through his company, Life Research International until 1971 when J. Hartwell, Chief of the Natural Products Branch in the NCI, advised Marin that he should contact the ARS as the NCI was relying more and more on the ARS for their procurement of plant samples. Most of the samples were extracted at the Wisconsin Alumni Research Foundation (later RALTECH). The isolation of active agents from plant recollections was assigned by the Chief of the Natural Products in NCI to chemists at the University of Arizona (Jack Cole), Arizona State University (George Petit), University of Illinois (Norman Farnsworth), Research Triangle Institute (Monroe Wall), University of Virginia (Morris Kupchan), Virginia Polytechnic Institute and State University (David Kingston), Purdue University (John Cassady), and others. The NCI terminated acquisition of natural products for antitumor screening in October 1982.

The major discovery during this period was taxol from stem-bark of *Taxus brevifolia.* Taxol (paclitaxel) is currently used to treat ovarian, breast and other cancers. The stem-bark sample that initially showed activity was collected in 1962 by an ARS botanist, A. S. Barclay, who was assigned (by Robert Perdue, Quintin Jones and Carl Erlanson, New Crops Research Branch) to collect in the western US. Barclay collected samples from more than 500 plant species from the desert southwest northwards, crisscrossing the state of California into western Nevada, north into

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[5] Tumors initially used in the screen were the L-1210 and two others randomly selected from a battery of 23 systems in which the sarcoma (SA) 180 and adenocarcinoma (CA) 755 were more frequently used (Abbott 1976, loc. cit.). Activity in the L-1210 (also LE) was very rare, about one in 12,000 according to D. Statz & F. Coon, *Preparation of Plant Extracts for Antitumor Screening*, Cancer Treat. Rep. 60: 999–1005, 1976 however, extracts from some plants (e.g., *Taxus brevifolia*) that were initially inactive against LE showed LE activity upon fractionation. Almost all plants that showed LE activity advanced to clinical studies (see also Perdue, J. Nat. Prod. 1982).

[6] By 1969, the ARS had supplied 19,000 samples; the first 10,000 were represented by 5,478 species in which 11% were confirmed active in one or more tumors (Perdue and Hartwell, *The Search for Plant Sources of Anticancer Drugs*, Morris Arb. Bull. 20: 35–53, 1969). By 1975, the NCI had screened ~67,500 plant extracts represented by 20,525 species—based on an actual count of all species in the NCI Plant Header File, orthographic errors and synonyms eliminated; 10.4% species were active (A. S. Barclay, *Distribution of Anticancer Activity in Higher Plants*, Cancer Treat. Rep. 60:1081–1113, 1976). By 1982, the NCI had screened ~120,000 extracts represented by ~35,000 species (Spjut 1985 loc. cit.). The ARS had supplied the NCI with ~58,000 samples in which about 10% were probably recollections and FOSI samples; the latter were assigned to the 80,000 number series

Oregon and the finally the state of Washington where he collected samples of *T. brevifolia* in Aug. 1962, a plant that he probably realized he had not yet collected by the red aril that develops in August. Barclay, who had several students assisting him in gathering samples, selected plants as encountered in the field based on availability in quantity of 500 g (dried), avoiding those he previously collected.

During the 22 years of NCI screening (1960–1982), the procurement methods, extraction procedures and bioassays employed became more selective in screening plants for new anticancer compounds. Although the NCI acquisition of plant samples was regarded random, and later bio-prospecting, plant procurement always had taxonomic guidelines (Spjut 1985, loc. cit.). Major changes in the screen included introduction of new tumor assays, extracting out tannins, and dropping bioassays that were sensitive to ubiquitous compounds. By 1969 two prescreen assays were primarily employed, the *in vitro* 9KB and *in vivo* P-388.[7] Inasmuch only one novel drug later emerged for treating cancer, more than 1,000 novel active compounds were probably discovered; 322 were listed by Hartwell in 1976.[8] It is interesting to note that in 1985, for example, of 3,500 new chemical structures discovered, 2,619 came from plant sources (Editorial, Science 247: 513, 1990). Additionally, many active compounds discovered by the NCI show apparent correlation with a broad spectrum of therapeutic uses of plants in folk medicine (Spjut & Perdue1976).[9]

Such a correlation between plants used in medicinal folklore and antitumor activity (Spjut & Perdue1976, loc. cit) does not mean that a search for novel drugs from plants should rely strictly on such criteria. Cancer is a disease that is not likely to be diagnosed correctly by a practitioner who lacks the appropriate training in modern medicine. Hartwell, however, compiled a list of 3,000 plants used against cancer, most of which are symptomatic of the disease.[10] Among ~1,200 genera known to occur in North America, Spjut found only five (5) that had not been screened by the NCI (October 1978), *Ayenia, Gratiola, Limosella, Malaxis,* and *Pinguicula*[11] (copy of memo in WBA RFP Tech. Prop. p.109), compared to 94 untested genera in a California flora (see WBA RFP Tech. Prop. p. 104A) that have no apparent history of medicinal use. This huge discrepancy is due to the fact that many plants reported in folk medicine are widely distributed, thus, will likely be collected in a systematic screening program (Spjut 1985, loc. cit.; see also worldbotanical.com). Moreover, the cost to collect samples based on a selective approach—that requires hunting down plants allegedly used in medicine—is at least 10 times that of a phytogeographical approach, or at least four times the cost for samples acquired by taxonomic guidelines employed by the ARS/NCI (Spjut loc. cit.). Anyone who has obtained both recollections and general samples for the NCI realizes the cost difference in these approaches.

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[7] In 1966, the NCI screen consisted of Walker intramuscular (WM) 256, LE and KB (Eagle) cell culture (KB). In 1969, P-388 leukemia (PS) was substituted for the LE and WM was dropped (Hartwell 1976, loc. cit.). The latter was sensitive to tannins, which by 1969 were being extracted out, and also phytosterols; both classes of compounds accounted for ~ 40% of all actives in WM (Perdue, ARS Cancer Program Guide, Oct-09-1970). Sometime around 1980, the Astrocytoma (ASK) was introduced to the NCI prescreen.

[8] J. Hartwell, *Types of Anticancer Agents Isolated from Plants*, Cancer Treat. Rep. 60: 1031–1067.

[9] R. W. Spjut & R. E. Perdue Jr., *Plant Folklore, A Tool for Predicting Sources of Antitumor Activity*, Cancer Treat. Rep. 60: 979–985.

[10] J. Hartwell, Plants Used Against Cancer. A Survey. Lloydia 30: 379–436, 1967, and 10 additional installments, ends with 34: 386–437, 1971.

[11] Spjut subsequently collected 1 kg samples of *Ayenia* and of *Gratiola* during 1978–1979. The remaining genera are tiny plants that were not practical to collect in quantity of 0.5–1 kg that was required for the NCI screen.

The chance discovery of novel antitumor agents from general screening—of mixtures of chemicals in (crude) plant extracts—depends more on the phytochemical diversity in the sample collection than on their ethnobotanical uses. Flowering plants (angiosperms) include ~225,000 species in ~15,000 genera, which is the baseline for assessing the distribution of chemical diversity in angiosperm species. From the genus perspective, then, the world flora is not all that large. From a statistical view, a sampling of the world flora might be based on major phytochoria, which reflect evolution and adaptive radiation in angiosperms. The flora of New Caledonia that contains 3,380 species, for example, is perhaps as equally significant as that of mainland United States which contains ~15,000 species. The greater chemical diversification at the family and genus levels is related to the early isolation of the angiosperm flora from breakup of the continental land masses and climatic changes that have since occurred on each land mass. The longer the geographical isolation of a flora, the more likely species will evolve unique chemical structures. Those that have had to adapt to increasingly drier climates will further evolve secondary metabolites that differ from their ancestral wet forest relatives. Spjut (1985, loc cit.), who recognized 58 floristic regions, suggested that the ideal collection of samples might be 500–1,000 species from each of Takhtajan's 12 floristic kingdoms. Perhaps, one might expect at least one new anticancer drug from each floral Kingdom (e.g., vinca alkaldois from Madagascar, taxol from NW North America, camptothecin from S China).

In 1986, the NCI redeveloped its natural products screening. Instead of a collaborative agreement with the ARS, the NCI awarded contracts to botanical institutions who had expertise in the geographic areas where collections were to be obtained, the Missouri Botanical was the primarily supplier for samples from Africa, the New York Botanical Garden supplied samples from the Latin America, and the University of Illinois at Chicago collected in SE Asia.[12] The NCI has obtained approximately 60,000 samples since 1986.

The new NCI antitumor screen with "60 different human tumor cell lines" became routinely operational in 1990 (Boyd and Paull, Drug Development Research 34: 91–109. 1995). However, the major novel discoveries so far are antiviral (Cragg et al. 1996).[13]

[12] Spjut had conveyed exactly such a scenario to the leader of the Plant Taxonomy Lab. Robert Perdue Jr., during the fall of 1982 when the ARS was reorganizing its laboratories. Spjut also had the understanding from the ARS management that he could continue collecting for chemists affiliated with the NCI program provided that his travel cost would be covered, Allan Stoner, Chairman, Plant Genetics & Germplasm Inst., memo to Spjut (Nov. 1982). However, Perdue, Program Leader in acquisition of germplasm of wild crops, later objected to Spjut being involved with plant collections for cancer research (Finney-Spjut comm., Mar-Apr 1983). The ARS then decided to support Perdue's objection. Subsequently, Spjut requested approval from the ARS to conduct exploration of plants for cancer research as an outside work activity in the newly created World Botanical Associates (WBA), May 1983. With the ARS approval, Spjut has since collected more than 5,000 samples from the U.S. and Baja California Mexico for drug discovery groups at various universities and the NCI. Spjut left the ARS in March 1997.

[13] Significant antiHIV agents discovered include (+)-Calanolide A and (-)-Calanolide B (costatolide) from *Calophyllum lanigerum* and *Calophyllum teysmanii*, respectively, in Sarawak, Malaysia; conocurovone from *Conospermum incurvum* in Western Australia; michellamine B from *Ancistrocladus korupensis* in Cameroon; and prostratin from wood of *Homolanthus nutans* in Western Samoa. Cragg, G.M., J.E. Simon, J.G. Jato, and K.M Snader. 1996. *Drug discovery and development at the National Cancer Institute: Potential for new pharmaceutical crops.* p. 554-560. In: J. Janick (ed.), Progress in new crops, ASHS Press, Arlington, VA.

Left: *Aleurites molucanna*, near trailhead, Honolulu Watershed Reserve, Ohau, photo by R. Spjut. This State Hawaiian tree, known also as kukui, was introduced long ago from Polynesia for the seed oil, used for burning lamps and in medicine, while the wood was used in the construction of canoes. We collected only seed. The major constituent is a common polyunsaturated fatty acid, linoleic acid. Other reported compounds include the phorbol diester 13-O-myristyl-20-O-acetyl-12-deoxyphorbol from wood, and flavinoids swertisin and 2"-O-rhamnosylswertisin. **Right**: “Cannon-ball tree,” *Couroupita guianensis* (Lecythidaceae), native to South America, cultivated at Foster Botanical Garden, Oahu, photo by R. Spjut showing fruit (amphisarca) along tree trunk, an example of cultivated plant that was seen only in botanical gardens; no samples were collected.

The Botanical and Chemical Significance of the Hawaiian Flora

Wagner, Herbst and Sohmer—in the revised edition of their *Manual of the Flowering Plants of* Hawai’i—indicate there are 956 native species, 91% endemic. Although Hawaii is well know for its endemic species, many of which are sensitive (including endangered or threatened), much of the vegetation that one sees is not natural. Indeed, it can be quite difficult to locate native plants. Why?

The Wagner-Herbst-Sohmer Manual treat, in addition to the native taxa (1,049 species, subspecies, varieties), another 861 species that were introduced to the islands, while as many as 4,600 introduced species may compete with the natives (Clifford Smith, Univ. Hawaii Website, http://www.botany.hawaii.edu/faculty/cw_smith/impact.htm). Introduced plants and animals, particularly feral pigs, are eliminating the natural flora. Even the best preserved state parks are not without exotic plants. The redwood tree, for example, a conifer indigenous to the Pacific Coast of northern California, seems out of place growing among native broad-leaved rain forest species in Kokee State Park, northwestern Kauai. In the Pu’u ka Pele Forest Reserve—where we were allowed to collect—the serious invaders are obviously species of *Eucalyptus* (bluegum, *E. globosus*), strawberry guava (*Psidium cattleianum*) and pines (*Pinus elliottii, P. taeda*), while the native species were limited in occurrence, mainly on steep slopes in ravines.

The exotic flora of Hawaii is undoubtedly chemically diverse due to introductions from many regions of the world. Many non-native tropical plants are also cultivated at botanical gardens in Hawaii. Such flora would seem ideal for the kind of antitumor screening conducted by the NCI program, especially since screening plant introductions do not require agreements with the countries of origin. However, the NCI has been obtaining samples from tropical Asia, Africa, and South America for the past 18 years. The more widely distributed species in these regions occur also on Hawaii and undoubtedly have been screened; indeed, ~⅓ have been screened since 1986 based on the NCI review of our proposed list of collectable species.

In preparing our list of proposed species, not all exotics were included, only those that the PI considered more promising for discovery of new anticancer drugs. An example of one that was excluded is candlenut tree, *Aleurities molucanna* (Euphorbiaceae). The PI recalled it was of interest for priority recollection during the 1970's. Stem-bark, leaf, and fruit of this species were active in P-388 leukemia from samples collected in India, and recollections were likely procured by the USDA Agricultural Research Service (ARS), Medicinal Plant Resources Lab. Additionally, woody stem of another related exotic species collected in Hawaii, *Aleurites fordii*, was active in KB; phorbol esters have been isolated from this species with known piscidal activity (Farnsworth et al. 1976).[14] The NCI has evaluated the pharmacology of Euphorbiaceae compounds based on anti-HIV activity in a Samoan sample of *Omalanthus (Homolanthus) nutans*, and species in other genera that have shown P388 and KB activity (e.g., *Croton, Euphorbia, Chamaesyce, Jatropha, Pedilanthus*). Their active agents—phorbol esters—have limited antitumor activity with carcinogenic potential (Farnsworth et al. loc.cit.; Suffness & Douros 1979).[15] *Aleurites molucanna*, which is common in ravines on the islands we visited, is easily recognized from afar by its pale whitish green foliage. Since we did encounter seed that was readily available, a sample was obtained because novel active agents may still be found in seed, which are generally more active than other plant parts, while also collected less often for the NCI screen.

We emphasize that we could have easily sampled other parts of *Aleurities molucanna* to increase our overall total of samples at a lower cost per sample, but in the interest of finding new anticancer drugs, we collected only seed from this species. It might be further noted that phorbol esters are often reported from family members (Euphorbiaceae) that produce a milky sap (Vogg et al. 1999),[16] and that not all active phorbol esters are tumor promoting.[17] An unpublished report indicated that P-388 and/or KB activity was generally distributed throughout the subfamilies and tribes.[18] *Codiaeum variegatum* is an example of Euphorbiaceae that lacks a milky latex and phorbol esters (Vogg. et al., 1999, loc. cit.), and has shown P-388 activity in samples from Costa Rica, Ecuador and Australia; a recollection from Hawaii was assigned to Jack Cole for isolation of active agents. Another example is a glycoside, phyllanthoside, first isolated by Kupchan from root of *Phyllanthus acuminatus;* it has undergone clinical studies for treating cancer.

In our proposed plant collections that were reviewed by the NCI to determine which have been screened (since 1986), very few **native** species were screened. The list of potential Hawaiian species for collection also indicated the number of collectable samples for each species and the actual number that had been extracted. For instance, the PI suggested five (5) samples

[14] N.R. Farnsworth, A. S. Bingel, H. S. Fong, A. A. Saleh, G. M. Christenson & S. M. Saufferer, *Oncogenic and Tumor-Promoting Spermatophytes and Pteridophytes and Their Active Principles*, Cancer Treat. Rep. 60: 1171–1214, 1976.
[15] M. Suffness and J. Douros, *Drugs of Plant Origin*, Methods of Cancer Research 26:73–125, 1979.
[16] G. Vogg, E. Mattes, A. Polak, & H. Sandermann, Tumor Promoters in Commerical Indoor—Plant Cultivars of the Euphorbiaceae, Environ. Health Perspect. 107: 753–756, 1999.
[17] Gustafson et al. *A Nonpromoting Phorbol from the Samoan Medicinal Plant Homalanthus nutans Inhibits Cell Killing by HIV-1*, J. Med. Chem. 1992: pp. 1978–1986.
[18] S. Saufferer, USDA ARS Economic Bot. Lab., 1981.

for *Pouteria sandwicensis* (Sapotaceae), a tree for which only one extract was listed, probably from a twig-leaf sample. We collected six samples: wood of root (wr), root-bark (rb), wood of stem with bark removed (ws), stem-bark (sb), tw (twig) and leaf (lf).

Permits

Inquiries for permits to collect plant samples in Hawaii were made to the Hawaiian Department of Land and Natural Resources, Division of Forestry and Wildlife, in Honolulu in October 2000, March 2001, and January 2003. In our response to the RFP, we proposed to collect in Hawaii during the years 2005 and 2006, but the NCI requested that we collect in Hawaii and other tropical areas sooner. Following award of contract in September 2001, we applied for permits to collect in Hawaii in January 2003.

Initially, the Hawaiian state authorities indicated that the appropriate permits could be obtained through the Honolulu office of the Division of Forestry and Wildlife; however, we had to apply for permits not only in the Honolulu state office but also in Kauai.

Included were letters of support from the Project Officer within the National Cancer Institute, Dr. Gordon Cragg, who pointed out that Congressional authorities had asked the NCI to include collections from Hawaii in their search for novel drugs from plant sources—presumably because of Hawaii's unique indigenous flora.

The island of Kauai has the richest flora in the Hawaiian Islands, and as one might expect the largest number of rare and threatened species (>90%). This was the major concern to Hawaiian authorities in granting us permission to collect samples for the NCI screen.

While protection of sensitive species is also our concern, we face monetary limitations in that we cannot afford to hire a native taxonomist and a specialist on sensitive species for every geographic region. "The State's overriding concern" has been "to protect and promote agricultural and other economically attractive interests" (Clifford Smith, Univ. Hawaii website). Our field team for Hawaii consists of one formally trained taxonomist, Richard Spjut, the PI, and his assistant, Richard Marin.

One specialist that might be of value in future collections, as suggested by one of our contacts on Oahu, goes by the name of "Rooster" who we heard lacks formal botanical training but knows the whereabouts of every individual for every sensitive species (rare, threatened and endangered species) in Hawaii.

The PI is reminded of a field specialist, Samuel Kibuwa, who assisted the USDA ARS leader of the Medicinal Plant Resources Lab, Robert Perdue, in collecting plant samples for the NCI in East Africa during the late 1960's. Samuel, like Rooster, also lacked college education, and additionally high-school education, but as a field assistant in plant explorations for the staff of the East African Herbarium, he had an uncanny ability to recognize species. In exploration of new areas where species were unfamiliar to staff botanists, Samuel often recognized them correctly to genus. Thus, he not only remembered the species characteristics (by characters he developed), but had the ability to classify them, and to recall their Latin names. In assisting Dr. Perdue, Samuel also could recall where they had previously collected a species; thus, Dr. Perdue was able to avoid duplication, although Dr. Perdue would duplicate collections of species from new geographic areas.

When the PI (Spjut) first collected in East Africa, he had asked Samuel Kibuwa to assist him in the field. However, Samuel wanted additional compensation from previous work with Dr. Perdue—who had paid him according to established pay standards. Samuel's demands—presented to the PI—included lodging at the highest rate hotels, per diem far beyond ours, and above standard wages. Since previous USDA Harvard Ph.D botanists (Quintin Jones, Robert Perdue) relied heavily on Samuel to accomplish their mission in East Africa, and that one other Ph.D botanist—who collected without assistance from a local taxonomist or Samuel—was unable to correctly identify many recollections needed for the NCI, Samuel and staff members at the East African felt certain that the PI had no choice but to hire Samuel. But the PI had had come prepared to work without Samuel, and the circumstances that had evolved, therefore, made it easy for the PI not to hire Samuel. The PI subsequently spent four months in Kenya obtaining recollections without any field assistance from the East African Herbarium, and as a result gained respect from taxonomists there by correctly recollecting nearly all the plant species that were assigned. When the PI left Nairobi, he and Samuel exchanged a friendly handshake. The respect Spjut earned is further evident from the assistance he received in the identification of the many general collections he obtained for the East African Herbarium with duplicates deposited at Kew and at the Missouri Botanical Garden.

Richard Marin has developed a keen eye for recognizing different species in the tropics even though he may not always know them by scientific name. He has done extensive ethnobotanical collecting with the Campa Indians in the Amazon and with other Indian cultures in Honduras and in Chiapas, Mexico as a student of Drs. Ernest Booth and Bruce Halstead. This is followed by more than 30 years of field experience in collecting flowering plants, marine algae, and invertebrates for the NCI and other pharmaceutical institutions. Consequently, Marin can readily recognize in the field the different species and plant parts needed for collection. Additionally, he has acquired a good familiarity with exotic crops such as avocado, loquat, guavas, etc by growing many of these himself. This knowledge was very helpful in Hawaii where one often encounters such plants growing singly in the midst of a mixed rain forest of introductions and native species. Marin was able to eliminate these species at a glance from distances of hundreds of meters. This allowed the team to readily focus on the native species that were of interest.

Marin, now, almost 60 years of age, is still quite agile. He is able to climb trees and reach the crown where branches often produce flowers and/or fruits that are necessary for identification. His tree climbing made it possible to collect samples of bark, twigs, and leaves from branches without causing harm to the tree. The Chief Forester at Maricao State Forest in Puerto Rico, who accompanied us almost daily in the field, frequently witnessed first-hand Marin's agility to move about in the tree tops.

In summary, the NCI has been fortunate to have Spjut and Marin as a team to collect samples. Spjut not only applies his taxonomic skills in the field, but also 34 years of knowledge of plant collections for the NCI in which he makes his best effort to find those that are new to the screen so that new anticancer drugs can be discovered. Marin not only has the ability and the good eyesight to locate a diversity of plants for Spjut to check out, but the physical strength and dexterity to obtain the kind of samples that are most likely to show anticancer activity. The work involved in the gathering and separation of samples is physically demanding and tedious.

Plant Parts

Plants are usually separated into samples of their different parts because active agents often concentrate in part of the plant. For trees and shrubs, this is usually in root, bark and fruit. Data to support this is presented below, preceded by data on plants used in traditional medicine, which is reproduced ±verbatim from a website by Ernest Rukangira:

The Ten most frequently used Plant Products by Traditional Healers in Tanzania

"1. *Cassis didymobotria L.*	**Leaves**	Anemia, Athlemintic, laxative
2. *Ficus stulhmanii Walp.*	**Stem bark**	Treats chronic wounds
3. *Harrisonia abysinica Oli*v.	**Roots**	Bilharzia, chronic wounds
4. *Terminalia serica Burch.*	**Roots**	Diarrhea, vomiting, stomach problems
5. *Securidaca longipenduculata*	**Roots**	Treats infertility in both men and women
6. *Euphorbia quadrangularis Pax*	**Arial parts**	General body weakness
7. *Entada abyssinica Steud.*	**Root bark**	Chronic cough, headache, stomach pains
8. *Albizia vesicolor Wel*w.	**Root bark**	Anemia, Athlemintic, sterility in women
9. *Strychino heterodoxa Gil*g.	**Roots**	Inflammations and fevers
10. *Gnidia kraussiana*	**Tuber**	Constipation, swollen stomach"

African Medicinal Plant Products Commonly Exported and Countries Involved

Apocynaceae		
Hunteria eburnea	**bark**	Ghana
Rauvolfia vomitoria	**root**	DRC, Rwanda, Mozambique
Strophanthus gratus	**fruit**	Cameroon
Strophanthus kombe	**fruit**	Italy (23%), Holland (13%), Germany (12%), France (11%), Spain (2.4%)
Tabernaemontana elegans	**seed**	Mozambique
Voacanga africana	**seed**	Cameroon, Côte d'Ivoire, France
Voacanga thouarsii	**seed**	Cameroon
Combretaceae		
Terminalia sericea	**bark**	Mozambique, Germany?
Euphorbiaceae		
Ricinus communis	**seed**	
Fabaceae		
Duparquetia orchidacea		Ghana
Griffonia simplicifolia	**seed**	Ghana, Germany, Côte d'Ivoire, Cameroon
Physostigma venenosum	**fruit**	Côte d'Ivoire, Nigeria
Liliaceae		
Gloriosa superba	**seed**	Mozambique
Menispermaceae		
Jateorhiza palmata	**root**	Tanzania
Pedaliaceae		
Harpagophytum procumbens	**root**	Namibia, Germany, Italy, USA, South Africa Mozambique, France, Botswana
Harpagophytum zeyheri	**root**	Namibia, Mozambique, Botswana
Ochnaceae		
Brackenridgea zanguebarica	**bark**	Mozambique
Rosaceae		
Prunus africana	**bark**	Cameroon, France

		Madagascar, Italy, Kenya, DRC Spain, Uganda
Rubiaceae		
Pausinystalia johimbe	**bark**	Cameroon, Holland (65%), Germany (18.3%), Belgium/Luxem-bourg (10.9%), France (5.9%)

The significance of active agents being concentrated in root and bark in the preceding tables is further evident in the table below for the frequency of NCI antitumor activity in P388 Leukemia and/or 9KB. Activity data are for woody plants classified by vegetation types, arranged in order from wet to dry types. These data were compiled by the PI during the mid 1970's from USDA accession records and collector's field notes and/or voucher herbarium labels, and the NCI Cumulative List of Active Plant and Animal Materials.[19]

Vegetation Types, No. Species Sampled, and Plant Parts Screened by the NCI

Woody Vegetation (#spp.)	Plant Parts And Percent Active KB and P388 Assays						
	rt/wr	rb	sb	ws	tw	lf	tw-lf
Tropical Rain Forests							
Amazon-Peru 932 spp.	2.8		5.0		2.5	2.0	
Africa-Ghana 107 spp.		3.2	5.7				1.9
Semi-dry Tropical forests							
Montane, Tanzania 169 spp.	3.7		8.7	5.2	0.5	1.2	
Lowland, Kenya 97 spp.	5.1		9.7	6.0	2.1	3.0	
Tropical Grassland/Woodland							
Montane, Tanzania 71 spp.	6.8		2.6	3.9	2.8	0.0	
Mediterranean/Desert Scrub							
Turkey, 597 spp.	5.1						3.7
California Desert/Chaparral							2.4

Data show that activity was most frequent in root and bark and least often in leaf. Generally, stem-bark is the most active plant part in tropical rains forests, except possibly for fruit. In drier

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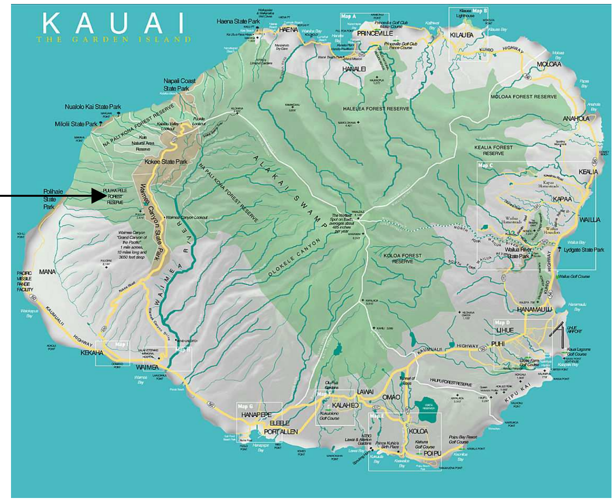
19 Data reported in USDA memoranda and presented at Scientific Meetings. Spjut, R.W. 1989. *Sampling plants for general screening of biological active agents*. Poster Presentation, Abstract, 13th Annual Meeting, American Society of Pharmacognosy, Aug 6-10, San Juan, Puerto Rico, P-26. Spjut, R. 1995. *A systematic approach to collecting plant chemical diversity*. Abstract. Oral presentation at the 36th Annual Meeting, Society for Economic Botany, Cornell University, Ithaca, Jun 21-25. Also published on worldbotanical.com. The percent active for California samples is lower than expected because collections of previously active samples were pulled out and submitted to Martin Jacobson for insecticidal screening.



Over-view of Kauai with arrow pointing to generally where most samples were obtained—in the Pu'u ka Pele Forest Reserve

vegetation types, the incidence of activity in root increases, while bark becomes less feasible to collect, especially in plants of Mediterranean and desert regions.

Perdue also conducted an independent analysis of his collections from East Africa. He reported on 94 active samples from Kenya in which 24 were root (rt, wr or rb), 18 were twig (tw), 17 were stem-bark (sb), 12 were wood of stem with bark removed (ws), 11 were leaf (lf), eight were woody stem with bark intact (ws-sb), and four were of other kinds.[20]

It might be noted that the inactive samples were extracted and tested a second time under a new extraction procedure with activity criteria changed to increase the number of actives, the P388 assay from %T/C of 130 to 125, and KB from ED_{50} (mcg/ml) ≦10 to ≦20.[21] For samples from Kenya and Tanzania, this increased the number of active stem-bark samples by 4-fold (400%), while other parts of the plant showed increases of 20-40%. Of additional interest is the antitumor activity in *Camptotheca acuminata* that was discovered in stem, leaf and fruit samples; Perdue et al. (1970, loc.cit.) reported that in106 samples of this species, 70% of the leaf extracts were negative in L-1210, while root was the most active.

Active Plants from Hawaii Prior to 1986.

The University of Hawaii supplied plant samples to the NCI during 1960–1982 (Hartwell comm., Spjut 1985, l.c.). A review of the active plants at that time provides an indication of what kinds of species were sampled (exotic vs. indigenous) and plant parts obtained, Appendix II. Of 93 active species reported, only 14% were indigenous. Many were herbs. Thus, many native Hawaiian species may not have been screened. Additionally, the exotic woody species screened were lacking in root, bark and flower/fruit samples based on a total of only 15 reported (~16%).

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[20] Perdue, R. E., Jr., *Procurement of Plant Materials for Antitumor Screening*, Cancer Treat. Rep. 60: 987–998, 1976.
[21] Perdue, R. E., Jr. KB Cell Culture. 1. *Role in discovery of Antitumor Agents from Higher Plants*. J. Nat. Prod. 45: 418–426, 1982.

Where are the Hawaiian indigenous Plants? The vegetation shown here is mostly introduced *Pinus-Eucalyptus* forest on ridges and slopes in NW Kauai, Pu'u ka Pele Forest Reserve. All green in the photos is largely species of pine and eucalyptus with a barren understory.

Kauai

Sep 09-30, 2003

State Permit: Aug. 12, 2003

The samples we collected from Hawaii are summarized in Appendix I, cited in order collected for our convenience since we are required to produce separate reports for each island. An asterisk denotes species introduced (exotic) to Hawaii.

For Kauai we collected 207 samples from 42 species. Most were from the Pu'u ka Pele Forest Reserve. We were also allowed to collect in the Lihu-Koloa forest Reserve, but this reserve was surrounded by private land for which we did not have permission to cross.

Kauai authorities kindly allowed us to stay at their forest facility in the Kokee State Park for a minimal fee, but our stay here was brief due to technicalities involving health insurance requirements.

The vegetation of the Pu'u ka Pele Forest Reserve is largely introduced. Plantations of mixed or pure *Eucalyptus* forest abound everywhere on slopes, while mixed pine (*Pinus taeda*, *P. elliottii*)-*Eucalyptus* forests occur extensively on ridges. The species of *Eucalyptus* are predominantly *E. robusta* and *E. saligna*. Extensive thickets of strawberry guava (*Psidium cattleianum*) are interspersed among the *Eucalyptus* forests, but there is no clear ecological pattern to any of the vegetation we saw. Stands of olive (*Olea europea*), *Albizzia*, *Acacia* ssp., and ironwood (*Casuarina equisetifolia*) were occasionally seen.

The forest under-story, especially near the margins, was generally dominated by *Lantana camara*, forming impenetrable thickets to ~ 1.5 m high in many places, although sometimes


Plants for Priority Screening. **Left**: Leaves of *Corynocarpus laevigatus*, (Corynocarpaceae), an invasive plant on Kauai, introduced from New Zealand, intentionally from seeds dropped from an airplane during the 1940's. Reportedly where trees of this species are removed, native species are not easily established, possibly because root toxins from *Corynocarpus* remain in the soil. The seeds are known to be poisonous. **Right**: *Pipturus albidus* (Urticaceae), a shrub about 1-2 m high that forms thickets in ravines. Leaves are used in folk medicine for treating cancer.

lacking in the pine forests. Other common species were *Dodonaea viscosa, Grevillea robusta, Styphelia tameiameiae*, and thorny bramble berries (*Rubus* spp).

From the aforementioned species, we collected samples only of the invasve *Psidium cattleianum* (*Spjut & Marin 15577*, Myrtaceae) and the indigenous *Styphelia tameiameiae* (*Spjut & Marin 15575*, Epacridaceae). The NCI showed no extracted samples for *P. cattleianum*, and two extracts for *Styphelia*, probably from woody stem with bark and twig-leaf; the additional samples of *Styphelia* are thus root and stem-bark (Appendix II). It might be noted that the fallen leaves of *P. cattleianum* are suspected of having an allelopathic effect on native species reproduction, while it is generally recognized that its growth habit in thickets shades out native species from ever becoming established from seed.

Other introduced species that were sampled are the invasive *Lophosetmon confertus, Melaleuca quinquenervia, Myrica faya, Passiflora ligularis*, and *Rhodomyrtus tomentosus.* Only *Rhodomyrtus* appears to have been previously collected. Our samples of the *Passiflora* vines included both root and fruit in which root probably has been rarely collected in this genus, or for that matter the family—Passifloraceae. *Passiflora v*ines tend to wind around lots of plants. Tracing the stems to the root in the under-brush is not always an easy task.

Other common species that were sampled are the indigenous *Acacia koa* and *Metrosideros polymorpha.* No previous collections were reported by the NCI for these species, which were frequently seen on ridges.

We obtained samples primarily from the three following areas:

> Pu'u ka Pele Forest Reserve, W of Waimea Canyon, E of Ka'aweiki ridge, 1000 m, 22º06'18.5"N, 159º40'57.4", Eucalyptus forests or *Psidium, Melaleuca, Pinus* forest with *Styphelia* in understory. *Spjut & Marin 15575–92*, *15599–600*.

Drying Plant Samples on Kauai. Heavy metal bins at Syngenta Seeds Inc. near Kekaha were used for drying samples in white cotton bags. Drying samples in this manner saved time and maintained sample quality since samples did not have to be removed from bags. Warm air was pumped into the bins up through the screens and vents, which then passed through the white cotton bags containing the sample material.

As above but mostly around Hunting Unit J, 3.5 mi post, 22º05'31.9"N. *Spjut and Marin 15593–98, 15601–09*.

Private land ~ 7 miles west of Lihue, *Spjut and Marin 15610-15*.

Of consideration for priority screening are samples of *Corynocarpus laevigatus* (Corynocarpaceae), *Pipturus albidus* (Urticaceae), and *P. kauaiensis*. The seeds of *Corynocarpus* are reportedly poisonous to mammals, while roots appear allelopathic—in producing compounds that inhibit germination of other plant species. We were unable to collect seed of *Corynocarpus*, but we did collect root and stem-bark samples. It is native to New Zealand, and was reportedly introduced to Kauai by aerial seeding.

Leaves of *Pipturus albidus* were presented to the PI by a resident on Oahu who indicated his wife employs the leaves for treating her terminal cancer (whom we also met). The Oahu plants were observed to have larger leaves, and were not collected there because we already had obtained a sample from Kauai where the smaller leaved plants were considered impractical for leaf separation, while we also had not yet been informed about the plant's use for treating cancer. Locher et al. (*Journal of Ethnopharmacology*, 49: 23-32, 1995) found anti-HIV activity in extracts of *P. albidus*. Another species in SE Asia, *Pipturus argenteus*, has been used for treating herpes. The bark, leaves and fruits of the Hawaiian species, known as Mämaki, have been used in folk medicine for treating high blood pressure, cholesterol, and other problems associated with the stomach, colon, liver, and bladder.

The *Wikestroemia* samples belong to a family—Thymelaeaceae—that has shown frequent antitumor activity; ~28% of the samples in this family were active in P-388 and/or KB.[22] The PI has recollected many species in this family such as *Dirca occidentalis* from California, *Gnidia glauca* and *Gnidia kraussiana* from Tanazania, *Gnidia latifolia* and *Gnidia subcordata* from Kenya. Active agents—that include gnidicin, gniditrin, and gnididin—are generally daphnane-type diterpenoids, similar to the phorbol esters of the Euphorbiaceae, the chemical name from

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[22] A. S. Barclay 1976, *Distribution of Anticancer Activity in Higher Plants*, Cancer Treatment Rep. 60: 1081–1113; J. Hartwell, *Types of Anticancer Agents Isolated from Plants*, Cancer Treat. Rep. 60: 1031–1067.

Invasive species on Oahu. **Left**: *Livistona chinensis* (Arecaceae), **center** *Trema orientalis* (Ulmaceae), and **right** *Schefflera actinophylla* (Araliaceae). *Trema* was not included on our list because the PI recalled this species was extensively screened prior to 1986. Samples were obtained from both *Livistona* and *Schefflera* because the NCI indicated no previous collections.

Daphne, a Mediterranean genus.[23] Daphnetoxin and mezerein are antitumor compounds isolated from *Daphne mezereum* that are also tumor promoters (Farnsworth et al. 1976, loc. cit.). The active diterpenoids have not shown activity in bioassays predictive for clinical development.[24] Species of *Wikstroemia* have shown P-388 activity from samples collected in Pakistan, Australia, Palau, Tahiti, and Hawaii. Three of the *Wikstreomia* species were assigned to Jack Cole who conducted fractionation with "good activity" results, and requested that high priority be given to *W. uva-ursi* for recollection from Hawaii.

We attempted to dry samples at our rental in Kekaha, but daily afternoon showers led us to seek a more effective solution. We contacted a Research Center Manger, Kevin D. McMahon, of Syngenta Seeds, Inc. after the PI had observed their facility located along the coastal Highway. Mr. McMahon not only made available Syngenta Seed's drying bins, but took the time himself to set these up for us to use. These dryers force warm dry air, generally maintained around 98˚ F, up through ventilated (screen) metal bin floors and then through the cotton bags containing the samples. The effect is similar to drying samples in the desert southwest on vehicle roof-racks while driving at highway speeds. Most samples dried within 3 days.

During the last day of drying on Kauai, we visited the Na Pali-Kon Forest Reserve (FR) where we observed many more collectable species than what we had collected in the Pu'u ka Pele FR. Had our permits included the Na Pali Kon FR, we estimate that we could have collected 400 samples in the three weeks that we spent on Kauai.

Upon shipping the samples from Kauai, we felt the local USDA airport authorities were overzealous in having to go through each of our samples to insure that no pest or diseases were present. Also, they seemed to have little regard for the value of the samples as they would discard sample material from each bag they opened. It took us two days to complete the inspection. Many leaf samples were initially rejected because of naturally occurring fungi on the leaves. USDA authorities on Oahu, however, were more considerate and had no problem in accepting similar samples for shipment.

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[23] S. M. Kupchan, *Novel Plant-Derived Tumor Inhibitors and Their Mechanisms of Action*, Cancer Treat. Rep, 115–1126, 1976.

[24] M. Suffness and J. Douros, l.c.; J. Cassady & M. Suffeness, *Terpenoid Antitumor Agents*; pp. 201–269 in *Anticancer Agents Based on Natural Product Models*, Academic Press, 1980.

Introduced Species near Tantalus Loop Trail, Honolulu Watershed Forest Reserve, Oahu. **Left**: Looking up at the crowns of tall trees of *Bischofia javanica* (Euphorbiaceae), a sole dominant of a tropical broad-leaved forest. **Right**: *Ficus pumila*, edge of forest near trail-head, an example of an introduced species not in our field identification guide; no samples collected.

Oahu

Oct 01-16, 2003
State Permit #27980

For Oahu we received permission to collect in the Waimano Public Hunting Area of the Ewa FR, and the Honolulu FR excluding the Nuuanu Public Hunting Area portion. We met with the Branch Manager, Patick G. Costales, of the Dept. of Land and Natural Resources in Honolulu who was very helpful in showing us on a map where we could collect. He further explained how far we would have to hike on trails before we would see native species. Mr. Costales also expressed interest in the NCI Letter of Intent.

Our Oahu collections were from Tantalus Loop area of the Honolulu Watershed FR:

> Leeward side of Koolau Mts., Manoa Cliff Trail off Round Top Drive, near Tantalus, 0.5-2.5 mi from trailhead, 500–700 m elev. Spjut & Marin 15617–26, 638–50.

Additional collections were obtained from private land as follows.

> Spjut & Marin 16527-37: from the Wainmanalo Forest Plantation
> Spjut & Marin 15651-53: from private residence at Kailua Beach.

Instead of the *Eucalyptus* and pines that we encountered on Kauai, we saw broad-leaved tropical species that to the untrained eye would appear as naturally occurring plants of a rain forest. But upon close examination, we could not find any native species.

One local dominant was *Bischofia javanica* (Euphorbiaceae). This species is apparently not classified as invasive in Hawaii; however, in southern Florida where it was introduced in 1947, *B. javanica* is considered a serious problem in displacing the native "cypress domes" and "tropical hardwood hammocks." The PI neglected to bring his data on the Hawaiian plants tested—maintained on his computer (left in his rental cottage)—the day he encountered the

Plants Used Against Cancer! Left: *Hibiscus furcellatus*, intentionally cultivated at the Wainmanalo Plantation for its medicinal properties, reportedly for treating cancer. Samples of root, woody-stem, stem-bark, and herbaceous stems with leaves were collected. **Right**: "Cancer Plant" or "noni," *Morinda citrifolia* (Rubiaceae), commonly cultivated in Hawaii; photo by R. Spjut from a plant at Wainmanalo. A "clinical summary" by Sloan Kettering (Web Site) states that "fruit, leaves, and roots of the tree Noni is a traditional Polynesian remedy for a variety of conditions including cancer, hypertension, and diabetes. Several polysaccharides, anthraquinones, and alkaloids are thought responsible for its activity. Animal studies show antitumor and immunomodulatory activity of noni juice extracts. University of Hawaii is conducting an NIH funded phase I study of noni in cancer patients." We did not collect samples of *Morinda* because the NCI indicated they had already screened 15 extracts.

plant, and because he was unable to recall anything about its bio-chemical screening, a decision was made to collect samples. Although collected elsewhere for the NCI, the NCI record number of extracts suggests it is of current interest; a stem-bark sample procured from Thailand by the USDA ARS Medicinal Plant Resources Lab was active in P-388.

The native plants on Oahu were much less accessible than on Kauai. As previously indicated, we had to walk considerably before seeing any native plants; most native species were collected ~1.0– 2.5 miles from the trail-head.

Of consideration for priority screening are samples of the introduced *Hibiscus furcellatus*. Rick Marin, during his regular Saturday off, met a Oahu citizen at Church services who, in the course of their conversation, mentioned a herb being cultivated on their plantation at Wainmanalo for its medicinal properties, specifically for treating cancer. We visited the plantation where the PI then identified the alleged cancer plant as *Hibiscus furcellatus*. None of the *Hibiscus* plants were in flower or fruit, but the leaf morphology seems to agree best with this species as confirmed by Roger Sanders. No extracts appear to have been screened of this species. We were also invited to make other collections on the plantation that we felt were of interest to the NCI; these included four species of ferns—*Blechnum appendiculatum, Phymatosorus grossus, Sphenomeris chinensis,* and *Nephrolepis exaltata*, one fern ally—*Psilotum nudum*, and four species of flowering plants—*Falcateria moluccana, Osteomeles anthellidifolia, Wikstroemia oahuensis,* and *Psydax odorata*.

Left: Thicket of *Freycinetia arborea* (Pandanceae), an indigenous species, on ridge ~ 1 mile from trail-head, Honolulu Watershed Reserve. Right: Drying bins at Garst north of Village Park showing the manager Paul Stuart in foreground and Richard Marin in back. We were fortunate to be able to use the drying bins, and Garst was generous in not charging us for the high cost of propane that is needed to run these.

We were advised by several independent sources in Hawaii of another "cancer plant," identified by the PI as *Morindia citrifolia* (Rubiaceae). No samples were collected of this species because the NCI indicated they had already screened 15 extracts.

Samples of *Coccoloba uvifera*, *Ficus microcarpa* and *Polyscias guilfoylei* came from a private residence where the owner wanted to eliminate these plants from his yard. The first two were not included on our list of proposed collections because the PI felt these probably have been collected for the NCI from other tropical areas; however, in recognizing the value of root collections, and that much of our digging work had already been done by the owner of the residence, we decided to take the readily available root.

Polyscias (Araliaceae) was not listed because it was not mentioned in our plant identification guidesl. This is one of the problems we had in collecting exotic species. Besides our field manuals lacking information on exotic plants, it was time-consuming to review each encounter to determine whether it has been collected for the NCI. Another exotic not in our field manual was *Ficus pumila* found growing near the Tantalus trail-head. No samples were collected of this fig since it belongs to a genus that undoubtedly with numerous species previously screened.

We have discussed with the NCI the possibility of including in our computer file their plant collection data on screening tropical plants so that we can review on site what species and parts have been previously collected and then make a decision as to what should be sampled. While it may be feasible to review such data, the cost to rely entirely on selective sampling is not within our present budget. This type of approach might be pursued under another type of reimbursable agreement. Currently, the most economical approach is to collect samples of the indigenous species, assuming that we are given the permission to collect in the forest reserves where these occur.

Samples collected on Oahu were mostly dried at Garst, a seed company specializing in corn. The Hawaii Station Manager, Paul B. Stuart, was very helpful in providing corn dryer bins and setting these up for us to use. He also did not charge us for the drying. Thus, drying samples in Hawaii was more economical than anticipated, and this helped compensate for the fewer number of samples collected than proposed.