REVIEW OF PLANTS COLLECTED FOR ANTITUMOR SCREENING

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ABSTRACT

Plant collection strategies for antitumor and anti-HIV screening are reviewed, largely based on collections for the National Cancer Institute during three periods: (1) 1947–59, (2) 1960–82, and (3) 1986–2004 (2008-). The primary strategy has been taxonomic collections of species (biodiversity) in geographical areas defined by political boundaries with consideration to phytogeography based on distribution patterns of genera. Antitumor active species are further reviewed according to plant parts and vegetation types sampled. The most significant antitumor compounds, including derivatives, are those employed in chemotherapy, or are currently in clinical trials. In the first period, in which ~1,500 species were screened, are colchicine from *Colchicum autumnale*)—previously known to be a mitotic poison, podophyllotoxin isolated from a crude extract (podophyllin, *Podophyllum peltatum* root-rhizome)—employed in folk medicine as a cathartic but investigated for cancer chemotherapy based on alleged use in treating venereal warts, the vinca alkaloids (vincristine, vinblastine) discovered from screening extracts of *Catharanthus roseus* based on folk use in treating diabetes, and the antileukemic activity in *Camptotheca acuminata* from a random screening of 1,000 plant extracts that led to the discovery of camptothecin. The second period was largely taxonomic collections from ~35,000 species in which ~3,500 were active; 2,192 crystalline compounds were isolated, 64 of which were evaluated in tumor panels. Significant compounds (plant sources) discovered from this screening are: bruceantin (*Brucea antidysenterica*), combretastatins (*Combretum caffrum*), ellipticine (*Excavatia coccinea*, *Ochrosia moorei*), homoharringtonine (*Cephalotaxus harringtonia*), lapachol (*Stereospermum suaveolens*), maytansine (*Maytenus serrata*), nitidine (*Zanthoxylum gilletii*, taxol (*Taxus brevifolia*) and triptolide (*Tripterygium wilfordii*). Some of the compounds are still in clinical trials, originating from samples collected nearly 50 years ago. The third period includes anti-HIV screening as well as antitumor screening of plants. Anti-HIV screening has found calanolides (A and [-]-B costatolide) in *Calophyllum lanigerum* and *C. teysmanii*, conocurvone in *Conospermum* spp. aff. *C. incurvum*, michellamine B in *Ancistrocladus korupensis*, and prostratin in *Homolanthus nutans*. Several antitumor active compounds in clinical evaluation are semi-synthetic derivatives: perillyl alcohol and flavopiridol based on a flavone in *Dysoxylum binectariferum*, another based on a steroidal alkaloid in *Veratrum californicum*, cyclopamine, which has long been known to cause birth defects in livestock, and MDR inhibitors, perveilleines from *Erythroxylum pervillei*. Relationships between plants used in medicinal folklore and those active are discussed. Problems experienced in establishing plant collecting agreements are also discussed. Due to budget cuts in the NCI screening of natural products, it is suggested that novel antitumor compounds in the WBA samples—collected since the year 2000—may not reach drug status until the year 2050.

INTRODUCTION

The objective of the Natural Cancer Institute's (NCI) natural products drug discovery program has been to bring a broad spectrum of biochemical substances before a screen of selected cancer systems in order to methodically sift out those of potential value for cancer chemotherapy. Natural products screening covers the entire process from collection of organisms to the final evaluation of clinical trials of a new drug (Perdue & Hartwell 1969). For plants, samples are initially obtained in small amounts (500–2,000 g dried) from which crude extracts are prepared and submitted to a "prescreen." Extracts that produce a significant response in one or more bioassays are considered active (Geran et al. 1972; Boyd 1992; Boyd & Paull 1995); the species from which the active sample and extract were prepared is an active species, the genus an active genus (Spjut & Perdue 1976). Discovery of activity in a species may lead to literature review on the pharmacology of active compounds including those isolated from related species and genera (Suffness & Douros 1979). If unknown, "recollections" (5 kg or more) are obtained to isolate the active agent(s) through fractionation guided by bioassay activity (Cragg et al. 1996). Novel compounds with good activity are further evaluated against a panel of antitumor assays for clinical development (Goldin et al. 1974; Sieber et al. 1976; Suffness & Douros 1979). Screening also includes natural and synthetic products acquired through systematic worldwide literature surveillance and voluntary contributions (Suffness & Douros 1979).

The NCI screen also evolves in which changes in acquisition and screening lead to new discoveries from species previously inactive. This includes active compounds in parts of the plant not previously collected as well as those that vary in presence and concentration according to developmental stages of the plant, growing season, ecology, geographical location, and endophytic organisms (Spjut 1985; Zärate et al. 2001; Strobel et al. 2004). An example is an old extract from a sample of *Solanum umbelliferum* Eschsch. collected by A.S. Barclay in 1965 from southern California; it was inactive in the NCI screen (Walker 256, Lewis Lung, KB) but 30 years later demonstrated significant activity toward DNA repair-deficient yeast mutants in which solasodine, O-acetylsolasodine and solasodine 3-O-ß-D-glucopyranoside were active agents isolated (Kim et al. 1996). Another sample of the same species—collected by R. W. Spjut in Feb 1972—showed activity in P-388 Leukemia (Aug 1973, CPAM 1977) for which recollections were made and sent to Morris Kupchan in Aug 1975 (USDA ARS 1960–82), but no reports could be found on the active compounds in P-388, while Kupchan had earlier isolated ß-solmarine, solapalmtenine and solapalmatine from other *Solanum* species based on activity in Walker 256 and KB (Hartwell 1976). Another extract from a sample collected by Barclay in 1965—of *Eriophyllum confertiflorum* (DC.) Gray—inactive in 1960's screen—was later active in P-388 (Aug 1972, CPAM 1977); recollections led to isolation of germacranolides of which the most active was eriofertopin (T/C 167, P-388; Cassady & Suffness 1980).

The evolution of the NCI involves not only the prescreens for detecting and isolating antitumor agents but also clinical development of less toxic derivatives, a continuing process in the improvement of the effectiveness of anticancer drugs (Farnsworth & Kaas 1981; Boyd 1992; Cragg & Newman 2004; Newman & Cragg 2007).

Collection strategies are reviewed according to plant taxonomy (biodiversity, bio-prospecting) and use in folk medicine for three periods of screening: (1) 1947–1959 (Goodman & Walsh 2001; DeVita & Chu 2008), (2) 1960–1982, and (3) 1986–2004 (2008–). Taxonomic data will also be presented and discussed for antitumor activity according to plant parts and vegetations types. This will include samples collected by the WBA that were screened by chemists at universities as well as the NCI. This is followed by a discussion on the relationships between plants used in medicinal folklore and those active in the NCI screen, and on problems in justifying agreements based on traditional knowledge of plant medicine.

TAXONOMY (Biodiversity, Bio-prospecting, Random, Systematic) VS. FOLKLORE (Ethnomedical)

The main difference between these two approaches is that the taxonomic approach provides for a scientific (systematic) basis to assess relationships among the samples screened, whereas the ethnobotanical approach is limited in scope to the alleged therapeutic uses of plants, which may be known by a local (ethnobotanical) name, although upon collection a voucher specimen may be obtained and identified by scientific name (Hartwell 1976).

Taxonomy is the study (or practice) of classifying organisms into hierarchical levels of relationships based on similarities and differences. The relevant taxonomic levels to a botanical screening program are class, order, family, genera, species (Barclay & Perdue 1976) in which subdivisions are also recognized (e.g., subclass, subfamily, subgenus, subspecies or variety). The naming of plants is governed by the International Botanical Rules of Nomenclature according to six principles: I— Independence from zoological and bacteriological nomenclature, II—Application of names according to types (standards), III—Priority according to date of publication, IV— Only one correct name is allowed for a circumscribed plant as ranked, V—The scientific names have to be in Latin, and VI—Rules are retroactive unless expressly limited (Greuter et al. 2000). Natural product chemists and pharmacologists, particularly those who advocate "traditional medicine," often do not seem to appreciate taxonomy as a useful tool to the discovery of new anticancer drugs; see Barclay and Perdue (1976), and Wheeler (1997).

The NCI screening of natural products has always employed a taxonomic approach to acquisition of samples, at least at the species level. This is not to imply that the NCI strictly targeted plants based on a "chemotaxonomic strategy" (Suffness & Douros 1979; Iwu 1997, four methods to selection of plants for screening)—such as alkaloids that might be pursued (e.g., Li & Willaman 1968). Rather, plant samples have largely been procured by a systematic process of elimination through taxonomic identification of species, employing Latin (scientific) names, although in some cases the sample may be identified only to genus. This NCI methodology has been referred to as random screening (Barclay & Perdue 1976; Hartwell 1976; Spjut & Perdue 1976; Spjut 1985) with limitations to duplication initially imposed at the species level (Spjut 1985). The philosophy has been that any species could yield novel compounds useful for treating cancer, not just those used in folk medicine (Perdue & Hartwell 1969; Hartwell 1976; Suffness & Douros 1979; Spjut 2005). Geographical areas were often selected by political boundaries and travel costs.

The random taxonomic collection of plants has also been referred to as biodiversity screening, bio-prospecting, mass screening, or systematic screening (Spjut et al. 1992; Barton 1997; Iwu 1997; Frisvold & Day-Rubenstein 2008). These alternative expressions can have different applicable meanings. For example, biodiversity, the genetic and morphological variation that exists—within species (such as ecotypes, forms, varieties, subspecies) as well as at higher taxonomic levels—is often assessed in terms of species numbers (Wheeler 1997; Marshall & Hillman 2000); worldwide estimates of vascular plants range from 235,000 (Suffness & Douros 1979; Spjut 1985; Wheeler 1997) to 750,000 (Farnsworth & Kaas 1981) for which a systematic sampling procedure might select geographic areas based on differences in vegetation at the species level as well as phytogeographic patterns at the genus level (Spjut 1985). Bio-prospecting considers species variation and other factors, which may include ethnobotanical uses (Soejarto et al. 2005) , as encountered by the collector in the field; for example, a collector may obtain a sample of fruit or bark of a species that may not have been previously available. The various methodical approaches to plant procurement may also be viewed as systematic screening.

However, the objective to a systematic screening program—in search of the unknown plant chemicals that may be used in cancer chemotherapy—is to obtain the broadest chemical diversity for the least cost (Spjut et al. 1992), a methodology that employs taxonomic data from previous screening as it pertains to plant classification and nomenclature, phytogeography, ecology, pharmacology, chemistry, and plant parts (Spjut et al. 1992). Thus, an effective systematic collection of the botanical diversity depends on feed-back from previous screening to refine plant collecting strategies—that may further target the collection of plant parts, species, genera or families, as well as to avoid their collection (Barclay & Perdue 1976; Perdue 1976; Spjut 1985; Spjut et al. 1986, 1992; Beutler et al. 1989). Systematic screening as defined by Spjut et al. (1992) emphasizes taxonomic characters other than folklore, political boundaries, and species.

The terms biodiversity, bio-prospecting and random also apply to conducting plant explorations without requiring knowledge of the species identification at the time of collection, especially before the US Endangered Species Act of 1973. A voucher was prepared at the time of collection and identified later, after shipping the samples and returning to the lab. Plant taxonomists are trained to recognize plants to family and often to genus. Even though the species might not be known by name at the time of its collection, a person trained in plant taxonomy can more easily recall what was collected than someone who lacks knowledge of plant taxonomy. It is therefore obvious that a random but methodical sampling of the available botanical diversity can provide a large number of samples at a relatively low cost, compared to selective approaches based strictly on chemotaxonomy or medicinal folklore. Additionally, in collecting general samples for the NCI program, one may also recall where a species previously collected occurs at other locations in case recollections are later needed. The random taxonomic approach thus provides an investment to later recollections, assuming that the plant taxonomist who first collected the sample is asked to make the recollection—and because of his or her previous experience with the initial collection, there is expectedly a lower cost in planning and in field surveys for its recollection.

I. Plants Screened for Antitumor Agents 1947–1959

The first period (1947–1959) is one of realization that plants are a major source of novel active compounds (Schepartz 1976), a result of a culmination of independent studies on experimental screening of extracts from many species according to specific therapeutic uses in folk medicine and from random acquisition. Plant extracts were part of a chemical mass screening arena of that included microbial organisms, fermentation products and synthetic compounds; for example, Boyd (1992–Table 1) classified 62 anticancer drugs in five groups: alkylating agents—11, antimetabolites—7, natural products and derivatives—12, synthetics—11, hormones and steroids—21, in which only three were from higher plants (vinblastine, vincristine, etoposide). Newman and Cragg (2007) and Cragg & Newman (2008) have since updated these reports and found that natural products, or their derivatives, are related to 63–70% of all new drug discoveries, while Mans et al. (2000) also recognized compounds from eight species of higher plants having a significant role in cancer chemotherapy.

Studies initially focused on specific plants or plant-derived compounds. In the early 1940's—known mitotic inhibitors were investigated such as colchicine (Ludford 1948; Bass & Probert 1950), which had been discovered in the 1800's from Autumn crocus (*Colchicum autumnale* L., Liliaceae)—based on its use in the medicinal folklore for treating gout—and other illnesses dating back to the first century in Dioscorides' *Materia medica* (Greene 1909); however, colchicine was toxic to normal cells due to the high dosage required and narrow therapeutic index (Suffness & Douros 1979; Babincova et al. 2009). Nonetheless, colchicine—and other cytotoxic compounds (e.g., maytansine)—provide skeletal models for finding less toxic analogs such as colchamine (natural analog)—used in Russia and elsewhere for treating skin cancer (CA 1962)—and for developing methods to shield the toxic effects such as by encapsulating colchicine and bio-chemically delivering it to tumor cells without damaging normal cells (Babincova et al. 2009).

Another cytotoxic compound, podophyllotoxin, was isolated by Hartwell and Shear (1947) from a crude extract known as podophyllin, employing the Sarcoma 37 assay. Podophyllotoxin had been earlier reported from this plant in regard to its use as an anthelmintic and cathartic (Millspaugh 1892). However, podophyllin (crude product), prepared from fresh root-rhizomes of May-apple (*Podophyllum peltatum* L., Berberidaceae), was investigated by Hartwell because of its use in folk medicine for treating venereal warts; later it was discovered that it had also been employed for treating cancer (Hartwell 1960, 1976). But it was not until much later that less toxic semi-synthetic derivatives were discovered—etoposide and teniposide—approved in 1983 for treating various cancers (Lee & Xiao 2005).

In the 1950's, a number of experimental studies were conducted on screening plant extracts against Sarcoma 37 based on specific therapeutic folk uses, taxonomic groups such as conifers and Amaryllidaceae known to include species used in folk medicine for treating cancer, and on plants with miscellaneous medicinal uses (Belkin et al. 1952a, 1952b, 1953a, 1953b; Taylor et al. 1952; Fitzgerald et al. 1953, 1958; Endicott 1957). These studies, which screened extracts from ~500 plant species, provided justification for further screening rather than new clinical leads. Actives included amoebicidal plants in the Simaroubaceae, *Simarouba amara*

Aubl. (also active against mammary adenocarcinoma C3HBA) and *Castela texana* (Torr. & A. Gray) Rose. Compounds isolated from later screening of Simaroubaceae in the 1960's and 1970's included bruceantin and holacanthone that underwent clinical trials (Suffness & Douros 1979; Cassady & Suffness 1980) but failed due to toxicity, while semi-synthetic derivatives continue to be investigated (Valeriote et al. 1998; Cuendet & Pezzuto 2004). The anthelmintic *Hagenia abyssinica* (Bruce) J. F. Gmel. (Rosaceae, syn. *Brayera anthelmintica*) in their survey on plants used to treat parasitic infections ("pesticides") "markedly damaged" Sarcoma 37, but samples of this species obtained in 1972 (*Spjut & Ensor 3070*, EA, HSC, K) from Kenya were inactive in the later NCI screen (KB, P-388 Leukemia). Many samples of Cucurbitaceae, Cupressaceae, and Liliaceae were active suggesting cucurbitacins, lignans, and saponins, respectively, compounds that have shown little potential for cancer therapy (Hartwell 1976). It was also reported that Hartwell had found podophyllotoxin in species of *Juniperus*. Other Sarcoma 37 actives—that are of interest today in alternative medicine—include St. John's wort (ethanolic extract, *Hypericum perforatum* L.), horehound (ethanolic extract, *Marrubium vulgare* L.), and Salmatian sage (aqueous extract, *Salvia officinalis* L.). Hartwell (1960) had received letters from Germany and Kenya recommending a Polish physician who had had achieved 'success' in ten hopeless cancer cases with an extract of St. John's wort, and while hypericinoids continue to be investigated as a cancer remedy—and in the detection of cancer (Mans et al. 2000), common side affects reported on the internet are hair loss, in contrast to an Australian patent filed in 1953 that included St. John's wort for hair restoration (Cruse 1959). Belkin et al. (1953) also noted that alkaloidal plants were mostly inactive, which included *Taxus baccata* L.

In 1955, the Chemotherapy National Service Center (CCNSC) was established as a service agency within the NCI to acquire and screen chemicals submitted by various outside researchers, and by 1958 had evolved into a targeted and integrated intramural drug development program (Zubrod et al. 1966; Schepartz 1976; Suffness & Douros 1979; Goodman & Walsh 2001).

Several important related discoveries in private industry soon followed, the vinca (indole) alkaloids, vincristine and vinblastine, currently used for treating Hodgkin's lymphoma, acute childhood leukemia, and other cancers. These compounds were isolated from the rose (Madagascar) periwinkle, *Catharanthus roseus* (L.) G. Don (Apocynaceae, a segregate genus of *Vinca*, synonym *V. rosea* L.), native to Madagascar and widely cultivated—and an invasive species—in warm regions (Codd 1963). The antileukemic activity was discovered serendipitously as a result of two independent research groups (the University of Western Ontario at Toronto and Eli Lily) extracting periwinkle samples in search of compounds for treating diabetes mellitus based on its reported folk uses against that disease (Carter & Livingston 1976; Sieber et al. 1976; Spjut & Perdue 1976; Goodman & Walsh 2001; Guéritte & Fahy 2005; Spjut 2005). During 1949–1955, their investigations had observed a decline in white blood cell count without a significant change in blood glucose levels in lab animals that were given extracts of periwinkle. Subsequently, one active component was identified by the university group (vinblastine) (Noble et al. 1958), while Eli Lily in 1958 discovered notable antileukemic activity in their anticancer screening program (P1534 Leukemia), which led to the isolation of vincristine (Svoboda 1961; Carter & Livingston 1976; Sieber et al. 1976). These anticancer compounds soon became established—in 1964—as useful drugs for treating cancer (Carter & Livingston 1976; Goodman & Walsh 2001).

Another important discovery was antileukemic activity (L-1210 Leukemia) from a leaf extract of *Camptotheca acuminata* Decaisne (Nyssaceae) screened by the NCI in 1959, which was among a random acquisition of 1,000 plant extracts that they had obtained in1958 from the USDA, who had earlier screened the extracts for cortisone precursors (Perdue 1968; Perdue et al. 1970; see also Correll et al. 1955). The USDA had found that their *Camptotheca* extract had tested positive for flavonoids, tannins, and sterols, but negative for sapogenins and also alkaloids (Perdue et al. 1970). The extracts were from plants established at US plant introduction stations (Chico CA, Miami FL, Savanna GA) for improving crop germplasm, for discovery of new crops, and for new introductions into horticulture (Hodge & Erlanson 1956). The plant samples were collected by the USDA in Sep 1951. Their extracts were subsequently placed in storage after testing for sapogenins.

The NCI discovery of L-1210 activity in *C. acuminata* was a good indicator of a clinically useful anticancer drug (Perdue 1968; Suffness & Douros 1979; DeVita & Chu 2008); however, additional plant material was needed to confirm the activity, but the species—a native tree to south-central China—was not readily available. Unlike the widely distributed periwinkle, relatively few plants of *C. acuminata* were available at the Chico station, and in southern California arboreta (Perdue 1968). Jonathan Hartwell, who had been in contact with the Agricultural Research Service (ARS) at Beltsville, Maryland for assistance on resolving nomenclatural problems in his study on plants used against cancer (Goodman & Walsh 2001), pursued recollections of *C. acuminata* and the procurement of general samples through a cooperative agreement with the ARS, established July 1960 (Schepartz 1976).

The USDA ARS—under a cooperative agreement with the NCI—obtained samples of twigs, leaves, and fruits of *Camptotheca acuminata* in Sep 1961 from the two plants at the Chico station (Perdue et al. 1970). The new leaf extract failed to confirm, whereas extracts from twig and fruit samples were active in L-1210 (Jan 1963). This led to cultivation of the species from which further recollections were later made for isolation of the alkaloid camptothecin (Wall et al. 1966). Camptothecin was thus discovered from a purely random screening of plant extracts. However, camptothecin failed in clinical trials due to toxicity. It was not until 30–34 years later that semi-synthetic derivatives, Topotecan (Hycamtin in 1996, GlaxoSmithKline) and Irinotecan (Camptosar in 2000, Pfizer) were developed and approved by the FDA for use against advanced ovarian and colorectal cancers (Rahier et al. 2005).

By 1960, the NCI had screened ~1,500 extracts from plants (Shepartz 1976), 1,000 of which were from the USDA extracts that had been screened in 1959 (Shepartz 1976) and another ~500 from the earlier screening by Morris Belkin and Dorothea Fitzgerald, in addition to ~115,000 extracts from fermentation products and synthetics (Goodman & Walsh 2001).

II. The NCI/USDA ARS Procurement of Plant Samples: 1960–1982

A major systematic effort to screen natural products began in 1960 when the NCI established cooperative agreements with various institutions (Schepartz 1976; Spjut 1985), the primary one for procuring plant samples was the USDA Agricultural Research Service (McCraken 1976; Schepartz 1976); it was renewed yearly for 22 years (Spjut 1985).

During 1960–1982, extracts from an estimated 35,000 species in 5,500 genera of plants were screened (Douros & Suffness 1981; Spjut 1985). The ARS Medicinal Plant Resources Laboratory (later Economic Botany Laboratory) procured ~58,000 samples in which ~4,000 were recollections. This included sequentially numbered accessions from 1–58,000 and another ~1,600 taxonomic special samples (FOSI and POSI, discussed later) starting from 80,000. The species estimate was extrapolated from a 1980 printout of all genera and number of extracts screened for each genus, and an actual count in June 1975 of 20,525 species in 4,716 genera, excluding synonyms as determined by Barclay and Perdue (1976). They also reported 67,500 extracts screened (from ~35,000 samples, Statz & Coon 1976) in which 2,127 species in 1,225 genera were active, in contrast to 77,382 plant extracts indicated by Schepartz (1976) for the same period (1960–1974); the ~10,000 fewer extracts in Barclay and Perdue (1976) may not have included those screened by other collaborators such as the University of Arizona (Tucson) and Chas. Pfizer and Co. (Maywood NJ) during 1957–1961 (Statz & Coon 1976); the WARF Institute (later RALTECH) was the major extractor for the NCI samples, Aug 1961–1982. The collection of plant samples, the extraction, the screening, and the isolation of active agents were all done under different contracts at different locations.

Before samples were shipped to the extraction facility, the scientific names for general samples accessioned by the ARS were reviewed for validity of publication based on use in floras and names listed in *Index Kewensis*. Plants that showed antitumor activity were further reviewed for consistency in nomenclature with other names in the Confirmed Plant and Animal Materials (CPAM) file and for identification of vouchers, deposited at the U. S. National Arboretum (NA).

Plant Procurement Guidelines

Although samples of species were collected as encountered in the field, plant taxa were also sought after on a limited basis, especially during 1971–75. Examples are the studies by Belkin et al. (1952 & seq.) and Fitzgerald et al. (1953, 1957), and the ARS procurement of **F**amilies **O**f **S**pecial **I**nterest (FOSI)— Amaryllidaceae, Apocynaceae, Celastraceae (including Hippocrateaceae), Liliaceae (*sensu lato*), Magnoliaceae and related families in Magnoliales, Rubiaceae, Rutaceae, Simaroubaceae, and Thymelaeaceae—and genera in other families such as *Colubrina* (Rhamnaceae, ansamacrolid colubrinol)—regarded as **P**lants **O**f **S**pecial **I**nterest (POSI). The FOSI were thought to be unusually rich in antitumor active agents (e.g., Apocynaceae with ellipticine, reserpine, tylocrebine, vinca alkaloids, voacamine; see also Raffauf & Flagler 1960), and along with POSI were targeted for new analogs of known antitumor compounds (Fitzgerald et al. 1958; Barclay & Perdue 1976; Suffness & Douros 1979). Instead of the usual 0.5–2 kg samples, larger samples, weighing 25 kg or more, were obtained; however, general samples of POSI and FOSI received through routine procurement were also included. The FOSI and POSI samples were assigned to a separate series of USDA accession (PR for Plant Record) numbers, the 80,000 series, and were forwarded to a fractionating chemist (e.g., Kupchan, Cassady, Farnsworth, Kingston, Wall) rather than to the routine extraction lab in Wisconsin (RALTECH or WARF [Wisconsin Alumni Research Foundation]). A special effort was also made to collect samples of conifers during 1969–1971 (Barclay & Perdue 1976) based on podophyllotoxin in Cupressaceae, taxol in *Taxus* and homoharringtonine in *Cephalotaxus*; ~90% of the known conifer species were eventually screened (Barclay & Perdue 1976). The

concept of FONI for **F**amilies **o**f **N**o **I**nterest was also suggested for grasses (Poaceae) and some related families (Barclay & Perdue 1976) but procurement never adopted this guideline because grasses are often time consuming to collect as evident by the fact that only about 12% of the genera were screened (Barclay & Perdue 1976).

Prior to 1979, species were precluded from further screening if previously active, or if 10 or more extracts had been tested without finding activity (NIH Memorandum 1971; Spjut 1985). It should be kept in mind that a species can be divided into plant parts from which either one or two extracts were prepared for each plant part sample; for instance, a tree divided into seven samples—(1) wood of root, (2) root-bark, (3) wood of stem, (4) stem-bark, (5) twig, (6) leaf, and (7) flower and/or fruit—could be represented by 7 or 14 extracts. During the early 1960's, an aqueous extract and an 95% ethanolic extract were prepared (Suffness & Douros 1979), later (Mar 1964–Apr 1974), a single solvent (aqueous/ethanol) procedure was adopted (Statz & Coon 1976); 100–150 g of pulverized dry plant material was extracted at room temperature by mechanical mixing in open beakers with 50% aqueous ethanol, then filtered and evaporated to yield a dry extract (Perdue & Hartwell 1969). Thus, a tree species might be collected once or twice before precluded from further screening, whereas herbaceous species, when sampled in whole, could be collected as many as ten times before being precluded from further screening (see Fig. 1 in Spjut 1985).

In 1979, limitations to the procurement of plant samples were also placed at the genus level. Genera were precluded from further screening based on pharmacology of known active compounds isolated, or by lack of activity in 100 or more extracts screened. Species duplication was further reduced to 6 extracts tested (instead of 10 extracts). The genus and species limitations were combined into a single listing known as SLOP for **S**pecies **L**ow **O**n **P**riority (Spjut 1985). This list was sent to all suppliers who were then asked not collect SLOP.

Extraction and Bioassays

Besides the limitations to collecting that had evolved, there were changes in extraction procedures and bioassays in which as many as 23 different assays had been in use (Abbott 1976). Initially, three bioassays were employed in the prescreen, Sarcoma 180, Carcinoma 755 and Leukemia 1210 (Hartwell 1976; Rettig 1977; Goodman & Walsh 2001). As feedback from screening of natural products was received, the extraction and bioassay procedures were modified as discussed further.

During the early 1960's, some assays were sensitive to ubiquitous compounds such as tannins that had no potential in chemotherapy (Perdue & Hartwell 1969; Hartwell 1976; Suffness & Douros 1979; Farnsworth & Kaas 1980; Cragg et al. 1996). Initially, tannins were extracted out before screening; later the bioassays that were sensitive to tannins and also phytosterols (Sarcoma 180, Carcinoma 755, and Walker 256) were dropped from the prescreen (Hartwell 1976). Screening of aqueous extracts was also discontinued. Several other bioassays such as the B16 Melanoma, new Lewis lung, and L-1210 Leukemia were retained for preclinical and clinical screening (Sieber et al. 1976).

The KB Cell Culture (KB) and the P-388 Leukemia (PS) were the major prescreen assays from1968–1979 (Abbott 1976; Suffness & Douros 1979; Farnsworth & Kaas 1981; Suffness et al. 1988). The KB assay is a culture of human cancer cells of the nasopharynx in artificial media in test tubes (Eagle & Foley 1958; Foley et al. 1958), whereas P-388 Leukemia is an in vivo assay, implanted in the peritoneum of selected strains of hybrid mice bred especially for the NCI cancer screening program. KB activity has been defined as an extract of 20 mg/ml or less that reduces 50% cell growth, whereas P-388 activity was determined by increase in mean survival, at least 25% , usually expressed as Test/Control x 100 (Abbott et al. 1966; Perdue & Hartwell 1969; Geran et al. 1972; Wall et al. 1976; Spjut et al. 1986); however, criteria for KB (and also P-388) activity were periodically tightened to reduce the number of actives (Perdue 1982).

It should be kept in mind that these were prescreens, employed not only for screening of crude extracts but for guiding fractionation and isolation of pure compounds in which further screening against other bioassays was also carried out in the development of novel compounds to anticancer drugs (Geran et al.1972; Goldin et al. 1974; Sieber at al. 1976; Wall et al. 1976; Douros & Suffness 1981). However, KB activity does not differentiate between killing normal and cancerous cells (Suffness & Douros 1979), and marginal activity in P-388 (25–50% increase in life span) was often due to sesquiterpene lactones (especially Asteraceae) and phorbol esters (Euphorbiaceae particularly Crotonoideae and Euphorbioideae, Beutler et al. 1989) that did not demonstrate activity in other bioassays (Hartwell 1976; Cassady & Suffness 1980). Nonetheless, the KB assay led to the discovery of taxol (Wani et al. 1971) in which fractionation of the extracts (fractions) of *Taxus brevifolia* Nutt. bark guided by this assay were found active in L-1210 (Perdue & Hartwell 1969), an assay predictive for developing a clinically useful drug (Perdue 1968; Suffness & Douros 1979), whereas the P-388 assay was known to detect over 95% of the clinically useful anticancer agents and not likely to miss activity in B16 Melanoma and Carinoma 38 (Spjut et al. 1986; Suffness et al. 1988). Subsequently, it may be noted that betulinic acid isolated from three unrelated species (Hartwell 1976), and known from many other plants (Cragg & Newman 2008), was inactive in KB, but active in Walker 256 (Hartwell 1976) and P-388 (T/C 140) that has since demonstrated activity in assays predictive for clinical cancer trials and against HIV (Cragg & Newman 2008).

In May 1974, the standard extraction was modified into a fractionation procedure that required more plant material, an increase from 500 g to 1.5–2 kg (Statz & Coon 1976; Suffness & Douros 1979; Spjut et al. 1986). Samples already in the pipeline and others that were inactive—represented by multiple plant parts of a species collection—were combined (ws-sb with tw-lf, sb with tw, rt with tw) to obtain sufficient quantity of material for extraction. This change in extraction procedure led to an increase in the number of active species, many of which were marginal in activity. Recollections, however, were prioritized based on tumor activity and history of activity in a genus with emphasis on P-388 activity: T/C>175 high priority (500 pounds), 150–175 medium priority (300 pounds) and low priority <150 (100 pounds). Plants active only in KB were assigned N.R. (No Recollection) status (NIH Memorandum 1978). One major discovery associated with this change is the combretastatins in twig-leaf samples of *Combretum caffrum* (Eckl. & Zeyh.) Kuntze; sodium combretastatin A–4-Phosphate has been in clinical trials since 1988 (Cragg & Newman 2005; Pinney et al. 2005). This is another example

of the length of time it takes for promising antitumor agents to reach clinical studies, 15 years since it was first collected in 1973, and is still in clinical evaluation—going on 40 years.

By 1979, the NCI had screened 108,830 extracts from approximately 35,000 species of plants in which they identified 4,712 active extracts represented by 3,286 species in 1,510 genera (Douros & Suffness 1981). Recollections discovered 2,192 crystalline compounds, 64 of which were evaluated in tumor panels (Douros & Suffness 1981). Within a year before termination of the NCI-ARS program, the NCI had screened 114,045 plant extracts in which 3,394 species in 1,551 genera were active (Suffness & Douros 1982).

III. The NCI Procurement of Plant Samples: 1986–2004 (2008-)

The NCI screening of plant extracts was placed in suspended animation in 1982 due to alleged lack of discovery of new anticancer drugs, although an estimated 3,500 species in 1,600 genera had been identified as active leads (Douros & Suffness 1981; Suffness & Douros 1982); however, this number is minor compared to 2,619 new chemical structures that were isolated from higher plants in just the year 1985 (Abelson 1990). The vinca alkaloids had reached anticancer drug status within 13 years of the plant's first investigation for use in treating diabetes, but this was largely before the "1962 Kefauver-Harris amendments" (Goodman & Walsh 2001)—requiring a greater degree of proof of effectiveness of a new drug before FDA approval, and also before the 1960 NCI/ARS cooperative agreement. The anticancer drug taxol, by comparison, took 30 years to attain drug status after the first sample was collected (Aug 1962–1992; Cragg & Newman 2005). As it went into Phase II clinical trials in 1985, the NCI re-established screening of plant products, initially for anti-HIV compounds. By 1990 a new antitumor screen consisting of 60 tumor cell lines was established (Boyd 1992; Boyd & Paull 1995). Instead of renewing their former contract with the ARS, the NCI, through competitive RFP's, contracted with the Missouri Botanical Garden, the New York Botanical Garden, and the University of Illinois in Chicago for samples from tropical regions (1986-2004), and the Morton Arboretum (1996–2000) and the WBA (2001–2004, and FY 2008) for samples from the United States and its Territories. Since 1986, the NCI has established a natural products repository of extracts stored at -20ºC in Frederick, Maryland. More than 200,000 extracts are kept at -20ºC in the NCI storage facility at Frederick MD. This along with the previous records of ~3,500 species of plants that have shown antitumor activity, particularly in the KB and P-388 assays, provide an invaluable pharmacological library.

The NCI plant collections were cut again in 2004 for the apparent lack of new drugs despite the promising compounds that were in the pipeline. These included older compounds such as bruceantin (*Brucea antidysenterica* J. F. Mill.), camptothecin (*Camptotheca acuminata*), homoharringtonine (*Cephalotaxus harringtonia* [Knight ex J. Forbes] K. Koch), lapachol (*Stereospermum suaveolens*), podophyllotoxin (*Podophyllum peltatum*), maytansine (*Maytenus* [*Gymnosporia*] *serrata* [Hochst. ex A. Rich.] R. Wilczek),), ellipticine (*Excavatia coccinea*, *Ochrosia moorei*), nitidine (*Zanthoxylum gilletii*, syn. *Fagara marcophylla*), and triptolide (*Tripterygium wilfordii*) that had failed phase I or II trials because of undesirable side affects but have since served as templates for development of less toxic derivatives, or have been reconsidered for adjunct chemotherapy or for other selective types of cancers. Taxol (*Taxus brevifolia*), which took nearly 30 years from the time the source plant was first collected, has

also included additional derivatives that are employed in chemotherapy (Hartwell 1976; Suffness & Douros 1979; Cragg & Newman 2006). As noted earlier, semi-synthetic derivatives of camptothecin and podophyllotoxin are now currently used in cancer chemotherapy.

New therapeutic compounds—discovered since 1986—are from the NCI screening of aqueous and organic extracts from ~ 65,000 plant samples represented by ~16,000 species (Beutler et al. 2006), and from pharmaceutical research on targeted compounds and screening in the NCI and private sector. The recently discovered antitumor compounds, or their semi-synthetic derivatives, in clinical studies include flavopiridol, a flavone derived from rohitukine found in *Dysoxylum binectariferum* Hook.f., a derivative from the naturally occurring cyclopamine—isolated in the 1960's from *Veratrum californicum* Durand because of toxicity to cattle and sheep (Keeler 1978; James 1999; Mann 2010), roscovitine derived from olomucine found in *Raphanus sativus* L and MDR (Multi Drug Resistance) inhibitors, pervilleines in *Erythroxylum pervillei* Baillon from Madagascar (Mans 2000; Cragg & Newman 2005, 2008). A number of discoveries have also been made from anti-HIV screening; these include calanolide A and (-)-calanolide B (costatolide) from *Calophyllum lanigerum* and *Calophyllum teysmanii*, respectively, conocurvone from *Conospermum* aff. *incurvum*, michellamine B from *Ancistrocladus korupensis*, and prostratin from *Homolanthus nutans*(Cragg & Newman 2005).

Although the discovery of cyclopamine dates back to the 1960's, a derivative has been developed for clinical trials only since 2004. Oncologists at John Hopkins University were looking for chemicals to target a specific embryonic developmental pathway, known as the Sonic hedgehog signaling pathway—related to a genetic (protein deficiency) disorder, "Gorlin's syndrome" that can lead to medulloblastoma. The researchers had recalled studies on the inhibitory effects of cyclopamine, and then found that cyclopamine did indeed suppress specific cultured brain tumor cells from mice and human medulloblastoma (Berman et al. 2002). A derivative of cyclopamine is under development by the Infinity Pharmaceuticals, Inc. (IPI) for treating various cancers associated with the Sonic Hedgehog pathway.

It is of interest to note that samples of *Veratrum californicum* that were active in the NCI 1960's screen probably did not contain cyclopamine (Spjut et al. unpubl.), and that subsequent samples that might have contained cyclopamine may have been precluded from screening by the NCI because of previously discovered antitumor activity due to other compounds. The occurrence of cyclopamine varies geographically and ecologically (Spjut 2010).

The numerous problems encountered to developing new anticancer drugs from a plant is generally described by Boyd (1992), and a detailed history is given for taxol by Goodman and Walsh (2001). Many plant-derived antitumor drugs have been recognized as mitotic inhibitors (Boyd (1992); however, their mechanisms of action vary as exemplified by camptothecin (topoisomerase I inhibition), 2-methyl-9-hydroxyellipticinium (topoisomerase II inhibition), vinca alkaloids (tubulin depolymerisation), taxol (tubulin stabilization), and bleomycin (DNA cleavage), while other types of mechanisms for remediation of cancer are evident by combretastatins (vascular disruption) and the MDR inhibitors.

VARIABLES IN PLANT COLLECTIONS

Plant Parts

Active agents are often concentrated in one part of the plant; however, plant parts collected for a screening program are also determined by weight requirements. Thus, while it may be desirable to collect stem-bark as shown below, such samples may not be practical to obtain from shrubs (Perdue 1976).

Despite the wealth of collection data for the NCI, studies on biological activity according to plant parts have been limited. One reason is that low frequencies in activity among the samples extracted require a large number of samples before convincing conclusions can be drawn. Another is that the procedural basis for sampling has never been standardized.

Perdue (1976) reviewed his collections obtained from Kenya for 44 species of woody plants that had shown activity in KB and/or P-388, his earlier study for herbaceous species collected in the United States (Perdue et al. 1970), and 3,472 samples ("extracts") from 1,041 woody species (based on the number of leaf extracts) collected by Sydney McDaniel and his associates near Iquitos, Peru; their collections yielded 89 active extracts from 67 species. Perdue's (1976) objective was to determine whether the same number of active species could have been identified if screening had been limited to fewer selective plant parts per species. In the case of herbaceous species, the authors concluded that it did not matter whether a separate sample of root was obtained (Perdue et al. 1970). For woody plants Perdue (1976) suggested that loss of active species could be minimized at a considerable savings in screening cost by just collecting stem-bark and twig in which a loss of 12 active species (27%) might be recoverable from screening related species elsewhere in Africa. Perdue (1976) also indicated that the active species in his Kenyan samples was most often in stem-bark followed by root. This conclusion was supported in the frequency (percentage) of active extracts by plant part samples from Peru: 4.5 % for stem-bark followed by 2.7 % for root, compared to 2.4% for twig, 2.2% for woody-stem (with bark) and 1.4% for leaf.

Spjut (1979, 1989, 1995) conducted a similar study that further evaluated antitumor activity according to vegetation types, reviewing samples collected by the ARS botanists and their subcontractors that included Perdue from Kenya and Tanzania, Enti from Ghana, McDaniel from Peru, Tosun from Turkey, and Spjut from southern California. Except for Spjut's collections from California, none of the samples were subjected to the exclusion screening guidelines. Perdue had collected in Kenya before Tanzania, and his Tanzanian samples were identified only to genus when submitted to the NCI screen. The McDaniel collections, which were identified to species, were the first from the Amazon Region. The large majority of the samples from Turkey had also been identified only to genus.

Spjut had field experience not only in California but also in Africa. He had visited the collection sites in Ghana and worked extensively in the areas where Perdue had collected in Kenya and in Tanzania.

In compiling data on Perdue's collections according to vegetation types, Spjut reviewed Perdue's field notebooks, and the determinations received from the East African Herbarium for his voucher specimens. Perdue's collections from the Southern Highlands of Tanzania, in particular, were perhaps the most comprehensive of any from a geographic area in numbers of species and in their separation of plant parts. This was largely the result of working closely with the natives and in having Samuel Kibuwa as his taxonomic guide for selecting plant species (Perdue pers. comm., 1973). Monetary rewards were offered to natives for finding species not previously sampled as determined by Kibuwa who also had retained a memory of what had been previously collected not only in Tanzania but in Kenya, a parabotanist who had years of field experience in working with professional taxonomists and was able to recognize all plants in the field, at least to genus. Similarly, the large number of samples from Peru provided a good representation of the botanical diversity in that region. The data for all of these collections are summarized as follows (Table 1):

Vegetation	Location	Rt	Rb	Wst	Sb	Tw	Lf	Tw-lf
Mediterranean Scrub	Turkey: 597 spp.	5.1 (231)						3.7 (597)
	California: 109 spp.							2.4
Tropical Grassland	Montane-Tanzania: 71 spp.	6.8 (59)		3.9 (52)	2.6 (38)	2.8 (71)	0 (70)	
Seasonally Dry	Montane-Tanzania: 169 spp.	3.7 (162)		5.2 (138)	8.7 (92)	0.5 (167)	1.2 (169)	
Tropical Forests	Lowland-Kenya: 97 spp.	5.1 (97)		6.0 (43)	9.7 (41)	2.1 (96)	3.0 (93)	
Very Wet Tropical	Amazon Peru: 932 spp.	2.8 (864)		2.3 (602)	5.0 (362)	2.5(871)	2.0 (932)	
Rain Forests	Lowland- Ghana: 107 spp.		3.2 (63)		5.7 (87)			1.9 (107)

Table 1. *General vegetation types and geographical areas indicating number of species (spp) sampled followed by percent active (KB and/or P-388 assays) of the total number of samples for each plant part.*
Rt = root, rb = root-bark, wst = woody stem that includes both woody-stem with bark (ws-sb) or without bark (ws), sb = stem-bark, tw = twig, lf = leaf and tw-lf = twig-leaf together.

Spjut's (1989, 1995, unpubl.) findings corroborate those of Perdue (1976) for stem-bark generally being the most active part of the plant followed by root. Additionally, a number of other interesting distinctions can be made. 1) Root was more frequently active in samples obtained from drier vegetation types. 2) Separating leaf from twig may yield twice as many leads in rain forest species (Kenya Lowland, Peru) than if not separated (twig-leaf combined, Ghana), whereas in samples from drier montane woodlands and grasslands (southern Tanzania) there

appears to be little or no value to separating leaves from twigs; here, it may be noted that woody species of the "Miombo" woodland are mostly deciduous. 3) Overall, the tropical forests with a well-marked dry season had the highest frequency of new active species. In southern Tanzania where vegetation varies from escarpment montane rain forests to plateau woodlands over short distances, the woodlands and patches of drier forests in ravines and on hilltops display a "pre-rain flush" (just before the rainy season commences). In coastal Kenya west of Malindi, a rich mosaic of lowland forests and scrub vegetation types occur as a result of convergences of present and past floras.

Many of Perdue's samples from the Southern Highlands of Tanzania that were inactive were extracted a second time under a new procedure (Statz & Coon 1976) and tested again in the P-388 and KB assays during 1977–78. Criteria for activity was reduced slightly (P-388 from 125 to 120, KB ED_{50} from 20 to 30; Suffness pers. comm. 1979). Additional actives, presumably marginal, generally doubled the frequency of activity for each plant part; for instance, 8.7% of the stem-bark samples from the montane seasonally dry forest in Tanzania that were active under the old procedure increased to 17.4% under the new procedure. A much greater increase—from 2.6% to 13.1%—was found for stem-bark samples from tropical woodland and grassland. Because it was not clear which samples were re-evaluated, and which parts were sometimes combined to obtain sufficient material for extraction (decided by RALTECH), data for the repeated extraction and testing were not included in the above table.

Also not included in the above table was a review of samples supplied by the Botanical Research Institute of South Africa, Pretoria (BRI) under the old and new extraction procedures. Under the old procedure there were 377 samples from ~150 species that were sent to WARF in 1972, but only three species were found active: *Uvaria caffra* E. Mey ex Sond. (ws-sb, tw-lf, both KB, Annonaceae), *Ficus sycomorus* L. (tw-lf, P-388, Moraceae), and *Combretum zeyheri* Sond. (lf, KB, Combretaceae). The relative paucity of active species appears related to the lack of root (4) and stem-bark (1) samples; their collection of other plant parts (and numbers) were: woody-stem—148, twig (tw)—6, leaf (lf)—37, twig-leaf (tw-lf together)—150, fruit—2, and aerial parts or whole plants of semi-woody to herbaceous species—29. Samples later supplied by the BRI—screened under a new fractionation procedure—yielded substantially more actives, nearly 90 species. The total number of samples and species screened under the new procedure were not tabulated because multiple samples from a plant often had been combined as mentioned earlier, but the active plant parts and their numbers for the woody species were as follows: ws-sb-tw-lf—31, ws-sb—14, tw-lf—8, and tw—1. Among the active species from samples not combined was *Combretum caffrum* tw-lf-fr from which combretastatins were later discovered. Samples of this species were randomly collected on two occasions, in Feb 1973 and in Oct 1974. Both collections were reported active, from ethanol, methanol and chloroform extracts, in KB and in P-388, the first in Oct and Dec 1976, and the second in Jul and Aug 1977 (CPAM 1977). The combretastatins were isolated from a 1979 recollection (Pinney et al. 2005).

The samples of California woody plants were largely aerial parts. Some species were precluded from screening because of previous antitumor activity in older tumor assays (e.g., *Asclepias albicans* S. Watson, *Crossosoma bigelovii* S. Watson, *Eriophyllum confertiflorum*); 2.4% of the species collected were found to be new actives in KB and/or P-388 (*Encelia californica* Nutt., *Hymenoclea salsola* Torr & A. Gray ex A. Gray, *Leptodactylon californicum*

Hook. & Arn.). Spjut has subsequently collected root and bark samples from the desert regions of Mexico (1979–1980) and the United States (1978-1980, 2001–2004, 2007–2008), and from Baja Californica (1986–2000) for screening at the Purdue University, Ohio State University, and later at the NCI (e.g., *Crossosoma bigelovii*).

The samples Spjut collected during 1979–1980 from the United States and Baja California are not directly comparable to that shown in the above table because of SLOP guidelines (Spjut 1985) and deployment of new assays. In 1980 the Astrocytoma (ASK) was substituted for the KB assay. Additional samples were also obtained from Baja California since the 1980 and were screened in vitro cancer cell lines by chemists at Purdue University and at Ohio State University. As in Perdue (1976) for Kenya, the distribution of actives according to separate and combined plant parts is summarized below for **40 active species** (Spjut and Marin 2000, Spjut 2003):

13—root only: *Atamisquea emarginata* Miers ex Hook. & Arn. ASK (Capparaceae), *Bergerocactus emoryi* (Engelm.) Britton & Rose ASK (Cactaceae), *Forchhammeria watsonii* Rose ASK (Koeberliniaceae), *Marina parryi* (Torr. & A. Gray) Barneby ASK (Fabaceae), *Olneya tesota* 1–5 human cell-line assays (Fabaceae), *Pachycormus discolor* Coville, 1–5 human cell-line assays (Anacardiaceae), *Parkinsonia microphylla* Torr. (syn. *Cercidium microphyllum* (Torr.) Rose & I.M. Johnston) KB (Fabaceae), *Phaulothamnus spinescens* A. Gray ASK (Achatocarpaceae), *Rhus integrifolia* (Nutt.) W.H. Brewer & S. Watson, 1-5 human cell-line assays (Anacardiaceae), *Stegnosperma halimifolium* Benth. ASK (Stegnospermataceae), *Stillingia linearifolia* S. Watson P–388 (Euphorbiaceae), *Viguiera deltoidea* A. Gray KB (Asteraceae), and *Xylonagra arborea* (Kellogg) Donn. Sm. & Rose P-388 (Onagraceae).

9—twig-leaf or stem-leaf only: *Acalypha californica* Benth. KB (Euphorbiaceae), *Berginia virgata* Harv. ex Benth. & Hook. f. ASK (Acanthaceae), *Bursera* sp. KB (Burseraceae), *Castela peninsularis* KB (Simaroubaceae), *Crossosoma bigelovii* (1–5 human cell-line assays (Crossosomataceae), *Dicraurus alternifolius* Uline & W. L. Bray ASK (Amaranthaceae), *Frankenia palmeri* S. Watson 1–5 human cell-line assays (Frankeniaceae), *Krameria erecta* Willd. ex Schult. ASK (Krameriaceae), and *Merremia aurea* (Kellogg) O'Donell ASK (Convolvulaceae).

3—stem-bark: *Esenbeckia flava* Brandegee KB (Rutaceae), and *Gochnatia arborescens* Brandegee KB (Asteraceae), *Jatropha cinerea* (Ortega) Muell.-Arg. (Euphorbiaceae), also active from root, P-388.

9—multiple plant parts: *Acanthogilia gloriosa* A. G. Day & R. Moran 1–5 human cell-line assays (Polemoniaceae), *Bursera microphylla* KB, *Callaeum macropterum* (DC.) D. M. Johnson (syn. *Mascagnia macroptera* (DC.) Nied.) ASK, *Castela polyandra* KB, P-388, *Dalea juncea* (Rydb.) Wiggins 1–5 human cell lines (Fabaceae, included by Barneby under *Psorothamnus emoryi* (A. Gray) Rydb., but recognized by Spjut as distinct from that species based on morphology and ecology, the combination in *Psorothamnus* was never made), *Hoffmannseggia intricata* Brandegee ASK (Fabaceae), *Salvia cedrosensis* Greene 1–5 human cell lines (Lamiaceae), and *Sphaeralcea axillaris* S. Watson ASK (Malvaceae). Seven species active in root, one also stem-bark.

5—whole plant: *Dyssodia anthemidifolia* Benth. ASK (Asteraceae), *Eriogonum preclarum* Reveal ASK (Polygonaceae) *Hermannia palmeri* Rose, 1–5 human cell lines

(Sterculiaceae), *Nama* cf. *hispidum* A. Gray,1–5 human cell lines (Hydrophyllaceae), *Orobanche cooperi* (A. Gray) A. Heller (Orobanchaceae).

1—flowers, but did not include samples from other parts, *Salvia mellifera* Greene (1–5 cell lines).

These results clearly support obtaining samples from separate parts of the plant whenever practical, 26 of 40 species, which excludes those with multiple parts active and samples of the whole plant; stem-bark and/or root were active in 32 of the 35 perennial or woody species.

As a final comparison, many samples collected by Spjut in Western Australia (WA) during 1981 were screened against KB in which activity in separate plant parts was again mostly root (Purdue Univ. 1986, unpubl. screening data received from McCloud and Cassady for 340 WA samples, PR-56531–56870, p.p.; Wall et al. 1987). Of 44 active species, 14 were active only in root, compared to just four (4) active only in aerial parts (excluding separate fruit samples). The remaining KB actives were samples of the entire plant or in multiple parts of the plant (Cragg ltr 1986; Purdue Univ. report 1986, unpubl; Wall et al. 1987). In one species of Restionaceae in which separate samples of the male and female plants were collected (of the whole plant), activity in the female plant (0.0359) was ~34 times more potent than that of the male plant (1.22). The Restionaceae are an ancient family of Gondwanaland distribution with no previous reports of biological activity until now (Spjut, McCloud, Cassady & Cragg unpubl.).

The most significant active that has emerged thus far from the screening of the WA samples is smokebush, *Conospermum unilaterale*, initially identified as *C. incurvum* according to the taxonomy at the time (before E. Bennett's taxonomic revision of the genus, unpublished ms of Bennett's revision provided to Spjut in 1991). This was not an anticancer active, but an anti-HIV active from which the novel concurvone was subsequently isolated (Decosterd et al. 1993). Concurvone was found to be entirely concentrated in root. Unrelated to this discovery is an L-1210 active of the whole plant, characterized by having well-developed root-rhizomes, which predates the Spjut collections from WA, and may still be of interest (Hartwell comm. 1974), especially since only 1 in ~12,000 samples screened were active in L-1210 (Statz & Coon 1976) and nearly all L-1210 actives from screening of crude plant extracts advanced to clinical trials.

Thus, when collecting samples from shrubs or perennial species with a well-developed root (including rhizome), separate root samples should be collected whenever practical to increase discovery of novel biochemical active agents. Although such plant parts may be viewed as destructive collecting, Spjut and Marin have demonstrated that such samples can be obtained from trees and shrubs without destroying the plant (WBA 2003 Annual report, http://www.worldbotanical.com/images/WBA-annual-rpt-yr3.pdf).

Extraction of the samples collected by the WBA for the NCI during 2001–2004 was completed in Jan 2009 (McCloud pers. comm, Jan 2009). The 2007–08 samples collected by the WBA were identified by the PI but a review of the identifications that was contracted for (with Botanical Research Institute of Texas, BRIT expired Dec 2009) has not been completed. Prior to 1981, reports of active species were received within 15 months of shipment to the extraction facility in Wisconsin. Since 1986, the NCI has done their extraction and screening in-house;

however, funding for the program has been cut a number of times, in 2004 and in 2008. Here it might be noted that although it took 30 years for taxol to become an anticancer drug from the initial collection by A.S. Barclay, the first recollection was made within just two months after first reported active (Jul 1964). As a result of the aforementioned NCI budget cuts, delays in identifications reviews, NEPA requirements, irregularities in permits being issued, and applying for patents, establishing CRADAs, and other administrative factors, one might project that it could take 50 years or more (year 2050) for any new drug discovered from the WBA samples to be approved by the Food and Drug Administration (FDA).

Common (Collectable) vs. Rare Species and Genera

Not all species are equally available for collection. Many are rare. Spjut (1985) estimated that 50% of a flora may be collectable; however, there is a diminishing returns in cost to collecting new species and in also maintaining the same level of taxonomic diversity at the genus level.

The availability of species is evident by comparing what has been collected within a flora of a defined geographical region to that which includes collection data from neighboring areas. California, for instance, was considered more collected than adjacent states; yet, within California only 27% of the available Rosaceae species were collected, whereas 51% of the same Californian species had been screened when data included samples from other states (Spjut 1985).

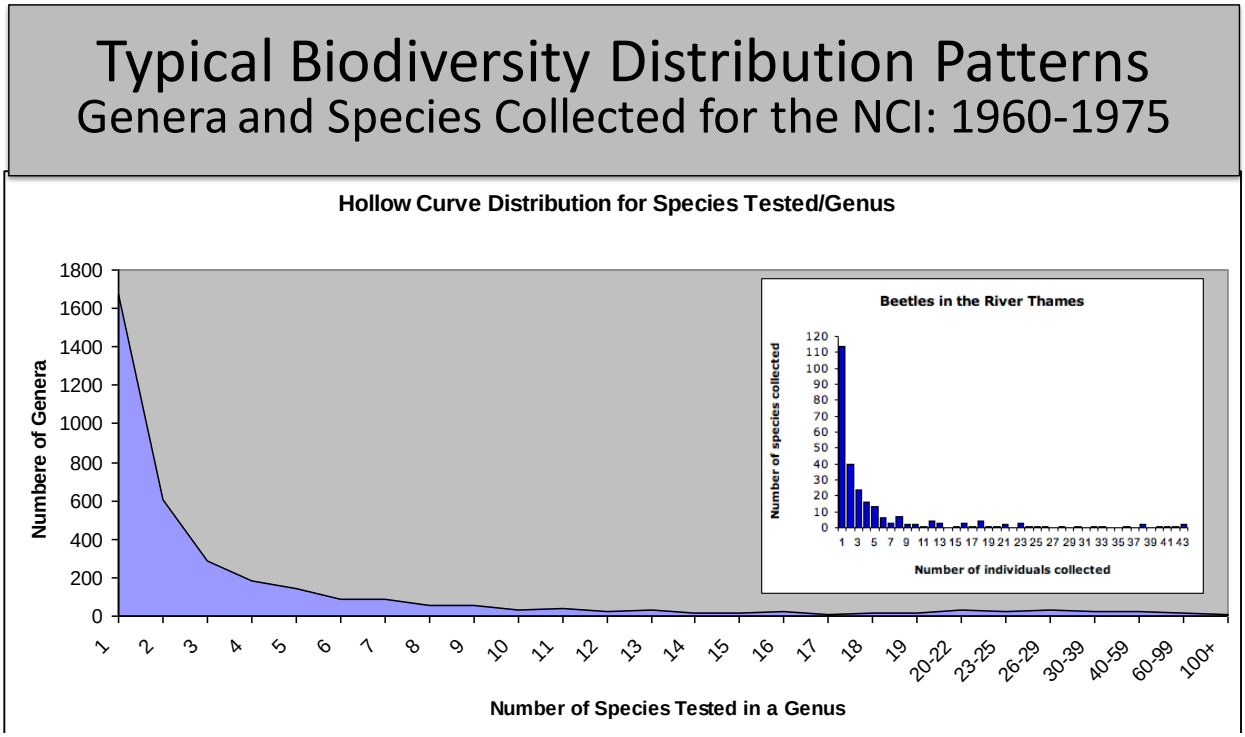

Fig. 1. *Comparison of numbers of species tested per genus collected for the National Cancer Institute, July 1960–June 1975 (Spjut 1985) with the number of individuals of beetles collected in the River Thames (Hubble 2001). These graphs illustrate the hollow curve distribution pattern (Willis 1922) as discussed in Spjut (1985). Perdue (unpubl.) found a yearly decline in number of KB active species that may be due in part to biodiversity distribution patterns.*

It should further noted that distribution patterns of genera and species are logarithmic, not linear as shown in a comparison of two graphs in the preceding figure (Fig. 1), one for the number of plant species collected per genus for the NCI (1960–1975, Spjut 1985), and the other on numbers of individual insect beetles for each species in a geographic region (Hubbell 2001). The graphs illustrate the "hollow curve" (Willis 1922) that characterizes biodiversity distribution patterns; the two curves are remarkably similar for two unrelated organisms.

As evident above, most genera collected for the NCI were represented by relatively few species: 1,672 genera by one species, 602 genera with two species, 289 genera with three species, 185 with four species, 146 genera with five species,...10 genera with 30 species,...to 5 genera with 100 or more species. At the other extreme, one-third of the species collected for the NCI program belonged to 201 genera, which in the above figure are mostly 16 or more species tested (Spjut 1985). Because weight requirements often require many individuals to be collected from herbaceous species, only the most common species usually get collected. Similarly, most species reportedly used in folk medicine have to be common to survive repeated collecting by indigenous people.

Based on field experience, taxonomic random collections from a geographic region began to decline notably in availability of species and genera not previously sampled after the first 20% have been collected (Spjut–Norris comm., 1981, 1990-1992, mosses collected in California). As one initially collects species from an area, most are monotypes (one species per genus), but as additional species are collected, an increasing number will belong to genera already collected; the diversity (number of species per genus) decreases in a hollow curve manner as shown above. However, one can start this process over again by going to a different geographic region, although a small percentage of the same species may be encountered, depending on whether the new region is on a different continent or corresponds to a high level of classification such as Floristic Kingdom (e.g., New Caledonia, Madagascar; see also Spjut 1985).

What are the Plant Geographical Regions?

Spjut (1982, 1985) divided the world into 58 floristic regions. An earlier version (unpubl., Spjut 1982) map is shown here from an unpublished report (Spjut 1982) for better display purposes; the Amazon (#17) was later divided into three regions (West, Central and East Amazon) while several other regions were combined. The boundaries drawn for the regions are based on distributions patterns at the genus level taking into consideration vegetation and other phytogeographic studies referenced by Spjut (1985) in view of the genus being the lowest level of chemotaxonomic diversity. The red colored regions were those most frequently collected, followed by partially collected regions in yellow and scarcely or not all collected in green. This can be compared to the following figure (Fig. 3) from Perdue (unpubl. but see also Perdue 1976) that shows the countries and states where plants were procured by the ARS and by the University of Hawaii during 1960–1975. Also, there were collections by the Commonwealth Scientific and Industrial Research Organization (CSIRO) from eastern Australian during the early 1960's that are not indicated on Perdue's map.

To further exemplify the significance of the phytogeographic regions, Spjut (1985) compared areas defined by political boundaries where collections had not been made in regard to

SLOP. It may be recalled that species were excluded based on number of extracts, not on the number of different locations sampled. This guideline (SLOP) correlated with species dominance in that species of trees would be excluded before those of shrubs and that shrub species would be would be excluded before species of herbs. Thus, when SLOP was compared to vegetation studies in Nicaragua, for example, where few collections had been obtained, 72% of the species that characterized the vegetation were SLOP, because of extensive collections made in nearby countries within the same phytogeographic region (Spjut 1985).

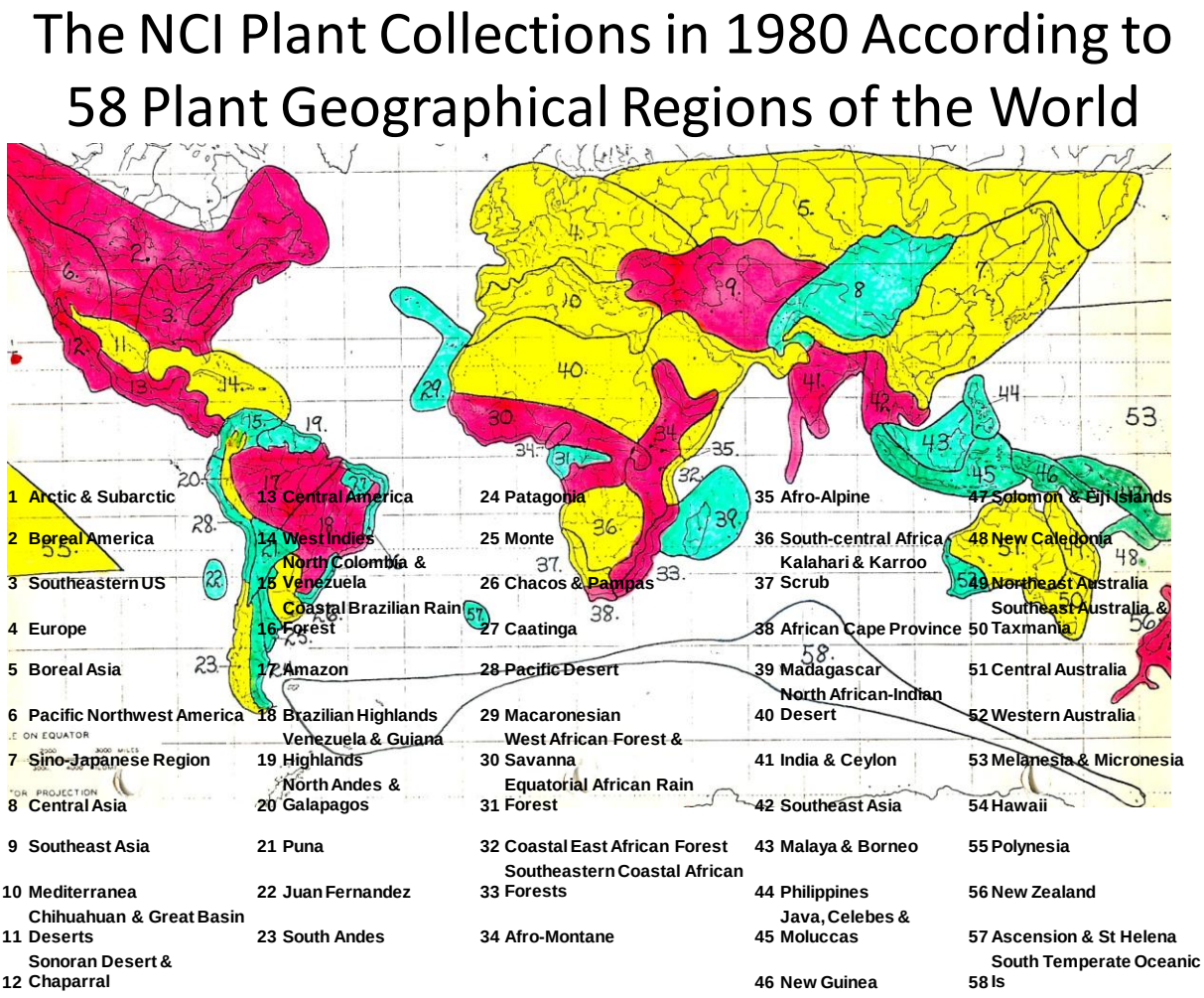

Fig. 2. *The floristic regions of the world as recognized by Spjut (1982, 1985) indicating where samples have been collected for the NCI; red is where many collections were obtained, yellow, fewer collections, and green, none or few collections, 1960–1982; see also Spjut (1985) and Fig, 3.*

From a phytogeographic point of view, the greatest diversity in the fewest number of plant samples might be obtained from areas where floristic kingdoms, subkingdoms and regions come together and where island floras have many endemic genera. Examples are 1—southwestern North America from U. S. to southern Mexico (e.g., California chaparral, Sonoran

Desert, Great Basin Desert, Chihuahuan Desert, southern montane coniferous forests, subtropical thorn forests, 2— Cuba (West Indies), 3—Venezuela (e.g., Amazon, Venezuelan & Guiana Highlands, West Indies, http://www.a-venezuela.com/mapas/mapaspdf/vegetacion.pdf), 4—Brazil (e.g., Amazon, highland forests, savannas, coastal rain forests, Caatinga), 5—Colombia, 6—Bolivia, 7—Chile, 8—Argentina, 9—Cameroon, 10—Congo, 11—South Africa, 12—Madagascar, 13—Mediterranean, 14—southwestern China, 15—Borneo, 16—New Guinea 17—Western Australia, 18—Tasmania, and 19—Hawaii. Political boundaries and loss of natural vegetation are not considered but nonetheless critical; see for example changes in forest over time—1945, 1960, 1974, 1990—in eastern Brazil at http://www.nybg.org/bsci/res/bahia/Defor.html. For centers of plant diversity in the New World, see Smithsonian Department of Botany: http://botany.si.edu/projects/cpd/samap.htm.

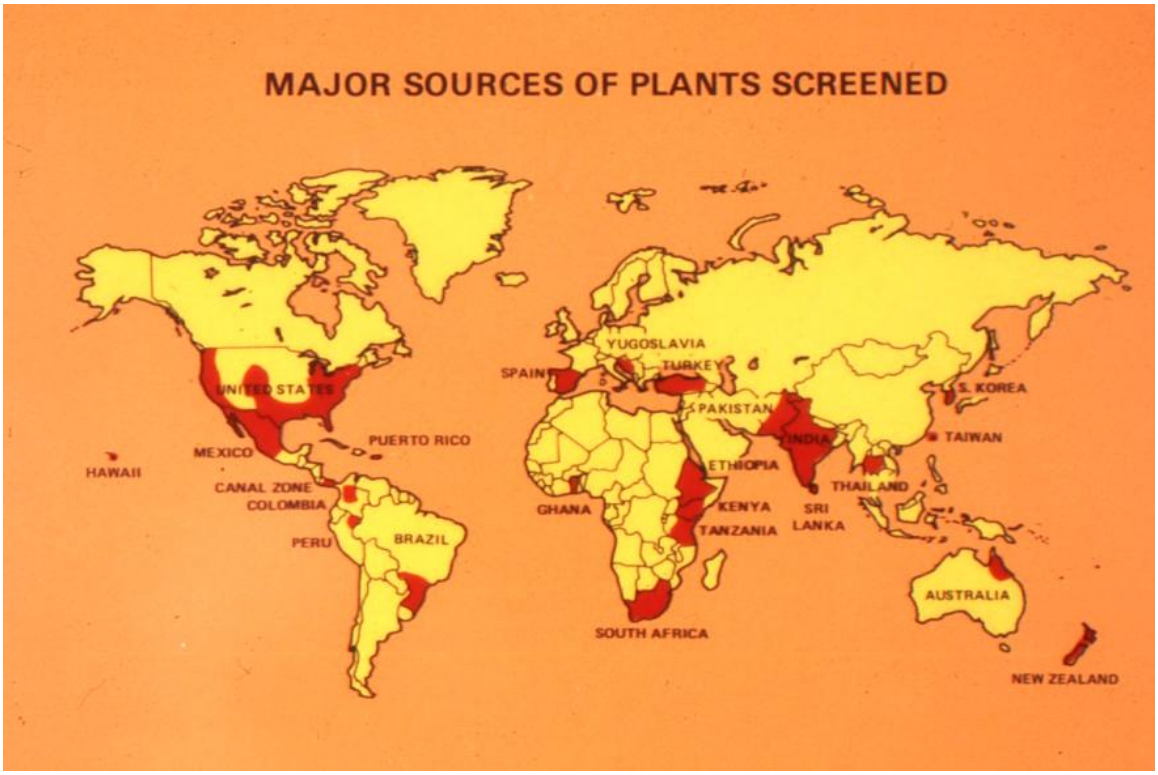

Fig. 3. *Geographical regions where the USDA ARS had procured samples for the NCI during 1960–1982 (prepared by Perdue in 1975; see also Perdue 1976).*

The distinctiveness of the island floras, particularly the New Caledonian flora, is evident not only in the endemic families and genera, but also in the paucity of species in families or subfamilies that are often collected in other biogeographical regions, namely Compositae, Poaceae, and Faboideae (Good 1964; Thorne 1969). Thus, the biodiversity on New Caledonia offers chemical novelty on a taxonomic level never before screened (by the NCI); however, up to 50% of the vegetation on this continental island, like many other places in the world, has been modified by human disturbance, dating back to 3,000 years, (Mueller-Dombois & Fosberg 1998).

MEDICINAL PLANT FOLKLORE

Medicinal plant folklore may be defined as plants used in medicine without substantiated scientific evidence as may be recognized by a legal authority, e.g., Food and Drug Administration (FDA). This includes "traditional knowledge" (or folk uses) of medicine whether written or unwritten, and many of the alleged botanical remedies sold as dietary supplements. The term medicinal plant is then confined to those from which medicinal compounds or drugs have been established (Farnsworth & Soejarto 1991).

Historical Record on Plants Used in Medicine

The historical records of plants used in medicine may be traced to the beginnings of agriculture (Hartwell 1960; Zohary & Spiegel-Roy 1975: Summer 2000); however, medicinal uses of plants probably coevolved with that of *Homo sapiens* as a hunter-gatherer, which dates back more than 10,000 BC (Vasey 1992; Gupta 2004), "a pattern of coevolution and mutual domestication between human beings and their various domesticates" that led to "adoption of techniques" "in which 'invention' played little or no role" (Cengage 2003). "Since many domesticates are plants that in the wild naturally accumulate around human habitation and garbage, and thrive in disturbed habitats, it seems very likely that the awareness of their growth patterns and the concepts of planting and tending would have been clear to any observant forager; thus, the techniques were not 'new'" (Cengage 2003). Moreover, many cultivated food plants have weedy relatives (e.g., amaranth, sunflower, beans, peas, wheat, rice, tomato) that were probably the easiest to select for cultivation because they were annuals that colonized disturbed habitats. Their uses probably spread via demic diffusion and cultural diffusion (Vasey 1992; Pinhasi et al. 2005).

The oldest records on plants used in medicine have been indicated to be 2800 BC with reference to the Chinese Emperor Shen-nung, the first herbalist (Perry 1961); the Pen Ts'ao—a pharmacopoeia on 365 medicinal plants (Summer 2000)—that included opium, ephedra, hemp, and chaulmoogra oil (from *Hydnocarpus* spp. for leprosy). This is thought to have been compiled under his direction ~ 2500 BC; however, the oldest known edition may be 50 AD (http://antiquecannabisbook.com/chap2B/China/Pen-Tsao.htm). Generally, the Sumerians are recognized for providing the first written records of medicinal plants, beginning around 2500 BC as evident from their "drawings of opium" (Simpson & Conner-Ogorzaly 1986).

The written accounts on medicinal plants is a continuous one over time. During the BC era are: *The Code of Hammurabi* under the direction of the King of Babylon (~1770 BC), *Ebers papyrus* from Egypt on 850 remedies (~ 1500 BC, Summers 2000), *Rig Veda* – the earliest Hindu text from Ancient India (~ 1500 BC) from which the Ayurvedic texts follow (*Charaka Samhita, Susruth Samhita* and *Ashtanga Hrdaya Samhita*) on 1,500 plant-derived medicines, Hippocrates (460–377 BC)—the "Father of Medicine," 300–400 species, Theophrastus (371–287 BC)—the "Father of Botany"—who gave instructions on the collection, preparation and use of 600 medicinal plant species in his *Historia Plantarum*, and Pedanius Dioscorides (1st century AD), a Greek physician who described 1,000 remedies in his 5-volume *De Materia Medica*, regarded by some as set back to the advancement of European medicine due to inaccuracies and lack of organization (Simpson & Conner-Ogorzaly 1986).

Publications on medicinal plants appeared more rapidly with the advent of the printing press in 1439 (Simpson & Conner-Ogorzaly 1986). Among the many European herbals that followed is the prevailing "Doctrine of Signatures" ("DOS," Bennett 2007) based on a religious notion that a plant's characteristic such as the color of the juice or shape of the leaf was to be used for a similar body fluid or part (e.g., lobed appearance of liverwort for liver ailments, red sap of bloodwort for blood disorders). This philosophy may originate with Pliny's *Natural History*, or may be traced further to Chinese medicine (Summer 2000; Bennett 2007); however, DOS may have been more of a taxonomic ("mnemonic") tool (Bennett 2007). The Chinese medical philosophy usually entails a compound prescription (of many species) pertaining to four different elements: (1) the principal curative effect, (2) an adjuvant for an increased effect, (3) the auxiliary to counter toxicity, and (4) the "conductant" for the target organ (T. Li 2009).

Limitations to Screening Plants Based on Medicinal Folklore

Plants reported for use in medicine are often not restricted to an indigenous culture (Spjut 2005); i.e. also to say that many compilations on medicinal plants include species based on reports outside the geographical area of study (e.g., *Medicinal Plants of the Philippines*, Quisumbing 1951). Whether any particular people can claim "traditional knowledge" of a species is questionable (Spjut 2005), especially in view of the historical accounts that represent an accumulation of knowledge on plants and their uses over time (Cengage 2003; Spjut 2005).

Farnsworth et al. (1985) reported that 74% of 119 plant derived drugs were "the result of chemical studies to isolate the active substances responsible for the use of the original plants in traditional medicine;" 45 of 121 in a later updated list were recognized as drugs in the United States (Farnsworth and Soejarto 1991). Their list of plant-derived medicines included caffeine, camphor, ephedrine, nicotine, tea, cocaine, tetrahydrocannabinol and others that may be regarded as dietary supplements in the United States. Also included in their tabulation are derivatives from the same or closely related species such as from *Anisodus tanguticus* (Maxim.) Pascher (anisodine, anisodamine), *Colchicum* spp., (colchicein amide, colchicine), *Digitalis* spp. (acetyldigoxin, deserpidine, deslanoside, l-dope, digitoxin, digoxin, gaianthamine, lanatosides), and *Papaver sominiferum* L. (codeine, morphine, papaverine, noscapine); thus, the actual number of species is less, "101", 36 of which are regarded as weeds (Stepp 2004).

Spjut (1985, 2005) recognized that a disproportionately large number of plant species used in medicinal folklore are widely distributed. For example, the historical *Medicinal Plants* by Charles Millspaugh (1892, also *American Medicinal Plants, An Illustrated and Descriptive Guide to the Plants Indigenous to and Naturalized in the United States which are used in medicine*), described in detail the preparations and medicinal uses for 180 species of which ~55 (30%) are weedy introductions from Europe. Moerman (2005) noted that 26% of the weedy species in North America are used medicinally by American Indians compared to just 8% of the indigenous flora. To further exemplify the extent to which a widespread species can be used medicinally—as a result of cultural diffusion (Spjut 2005)—is Moerman's (2005) example of the nearly ubiquitous yarrow (*Achillea millefolium* L.)—used medicinally "just about everywhere" with exception to Hopi Indians of New Mexico and the Tsimshian Indians of British Columbia. This European species was recently indicated to be native to North America, where it may have immigrated from Asia across the Bering Land Bridge (Ramsey 2008), possibly when *Homo*

sapiens migrated to North America at a time when its megafauna also began to decline (C. Johnson 2009; Gill et al. 2009). Among the indigenous species mentioned in Millspaugh (1892) is *Podophyllum peltatum* with references to the isolation of podophyllotoxin (Millspaugh 1892), although the precise structure was not determined until much later (Lee & Xiao 2005).

Many widely distributed species in folk medicine probably attained their distribution through trade just as food crops have found their way into many cultures. An example is guava (*Psidium guajava* L.), a food plant native of Central America commonly used in deserts and fruit salads (van Wyk 2005). It has been reportedly used in the Philippines (bark, roots, leaves) for washing ulcers and wounds, in Pakistan (stem-bark) for dysentery, in India (root-bark, stem-bark, leaves) for diarrhea and toothache, in Africa for diarrhea and malaria, in Mexico (leaves, fruit) for cleansing ulcers, and as an anthelmintic and for dysentery, in the West Indies a febrifuge and for dysentery, in Uruguay (leaves) for leucorrhoea, and in Costa Rica (flower buds) for diarrhea (Roig & Mesa 1945; Quisumbing 1951; Pakistan Forest Research Institute 1956; Watt & Breyer-Brandwijck 1962; de Montellano 1975). The various medicinal uses of guava have multiplied through cultural trade for the past several thousand years (see Raintree Nutrition, Inc, 1995– http://www.rain-tree.com/guava.htm), and like many other introductions, it has become invasive such as in Hawaii and Fiji (Wagner et al. 1999). Among Farnsworth et al. (1985) 101 species of drug plants are those that are consumed for food or grown as crops such as black mustard (*Brassica nigra* L.), areca palm (*Areca catechu* L.), pineapple (*Ananas comosus* (L.) Merrill), papaya (*Carica papaya* L.), tumeric (*Curcuma longa* L.), artichoke (*Cynara scolymus* L.), licorice (*Glycyrrhiza glabra* L.), cotton (*Gossypium* spp.), *Citrus* spp., and kava (*Piper methysticum* Forst.). Here guava might be added as "a plant drug from guava leaves (standardized to its quercetin content)" used for the treatment of acute diarrhea (Raintree Nutrition, Inc, 1995– http://www.rain-tree.com/guava.htm).

The greater the number of medicinal applications for a species the more likely it has been collected for the NCI screen (Spjut 2005); for example, 51 of 68 species specifying use for hemorrhoids in Quisumbing's (1951) Medicinal Plants of the Philippines were also reported for 11 or more medicinal applications for which at least 94% were screened from samples collected outside the Philippines (www.worldbotanical.com/Philippine_plants_tested.htm; Spjut 2005).

Although Farnsworth et al. (1985) have suggested that "25% of all prescriptions dispensed from community pharmacies from 1959 to 1980 contained plant extracts or active principles prepared from higher plants," it seems that actual case studies—where investigators pursed a specific ethnobotanical use against a particular disease that led to discovery of a new drug for that purpose—are not clearly substantiated. An example is the discovery of cardiotonics from *Digitalis purpurea* L. in 1775 by a physician in Staffordshire who had followed-up on one of his patients that had independently found a herbal remedy for his heart problem. The herbal remedy was a "concoction" of at least 20 different herbs in which foxglove was subsequently identified by the physician as the active ingredient (M. R. Lee 2005); the physician in this case was also knowledgeable in botany and chemistry. The legitimate question then is did the physician's source know that foxglove was responsible for the activity, or was it never really known for sure which of the 20 or more plants in the remedy was responsible for the activity. Moreover, *Digitalis* had an earlier history of being used as an ordeal poison, and for treating

dropsy (M. R. Lee 2005). Most FDA approved drugs discovered from plants with a history of use in medicine appear ex post facto (Fabricant & Farnsworth 2001).

Retrospective studies on comparing antitumor active plants used in folk medicine with those collected at random by Spjut and Perdue (1976) indicated that plants used in folk medicine were about twice as likely to show activity compared to those collected at random; however, data in this study did not adjust for the differences in the hollow curve distribution pattern of species between these categories (Spjut 1985, 2005). As indicated above, a greater proportion of the medicinal plants are more widely distributed than those collected at random (Spjut 1985, 2005). The correlation that Spjut and Perdue (1976) showed was largely due to cytotoxicity (KB assay); i.e. medicinal reports of plants indicating toxicity (e.g., anthelmintic, cathartic) were more likely to show activity in the KB screen (Spjut 2005) than medicinal plants in general. Indeed, plants used to kill fish or mammals had the highest percentages of active species (Perdue & Hartwell 1969; Spjut & Perdue 1976; Spjut 2005), which included known cardiotonic species (Farnsworth & Kaas 1981). Because the folklore plants are more widely distributed, they would also be expected to have a higher proportion of tannin and saponin actives (Farnsworth & Kaas 1981) than those represented in the random screen. The lower percentages of active species in Hartwell's (1967–1971) plants used against cancer, 17.3%, compared to 22.4% in Quisumbing (1951) medicinal plants in general was suggested to be the result of a larger number of species in Hartwell's (1967–1971) study, 2,725 compared to 855 in Quisumbing (1951). Data in Spjut (2005) showed that 66% of the Hartwell's (1967–1971) plants had been screened compared to 73% in Quisumbing (1951).

These retrospective studies cannot possibly account for the various uses and methods in preparations, especially when the plant samples are uniformly extracted in the NCI screen. First, it must be realized that ethnobotanical preparations are not pure compounds. Whether it is the whole plant, or part of the plant, or a crude extract, it is a mixture of many chemicals (crude product). Second, the preparations are not uniform (paste, tea, cold infusion, hot infusion, inhaled, smoked, consumed internally, etc.). Third, the plants employed may be only part of a complex preparation of many plants and other substances as often the case in Chinese medicine. Then there is the placebo effect that to some extent is part of cultural evolution of medicinal folklore ("psychological effect," Suffness & Douros 1979). Undoubtedly, the species that get selected over time—through trial and error—are those most likely to effect a physiological change. The question that scientists investigate is whether the physiological change is related to a **new** chemical that can be developed as a drug and/or whether it is the application or the crude product itself that is unusual.

The NCI screening program identifies specific (pure) compounds through fractionation guided by assay results, and it is the pure compounds that are employed in cancer chemotherapy, not the crude product, although several related pure compounds may be employed in combination chemotherapy(Carter & Livingstone 1976; e.g., etoposide and teniposide; see also Boyd 1992 and Newman & Cragg 2007).

A case in point is the NCI investigation of prostratin (a polar 12-deoxyphorbol ester) from a preparation of *Homalanthus nutans* (G. Forst.) Guill. (Euphorbiaceae). The compound had been isolated from this species for its anti-HIV activity, but it was not a new compound. It

had been discovered earlier by Cashmore et al. (1976) from an unrelated species, *Pimelea prostrata* (J. R. Forst. & G. Forst.) Willd. (Thymelaeaceae) that was investigated for its toxicity to livestock (St. George's disease) and for its antitumor activity. *Pimelea prostrata* was active in P-388 leukemia (Mar 1973) based on a leaf sample collected by the ethnobotanist, George Uhe, from New Zealand in Apr 1972. Daphane orthoesters and "P-factor" were also isolated from a recollection of the whole plant obtained in 1979 (Petit et al. 1983). Prostratin, which was not reported active in P-388, but nonetheless a derivative of the tigliane diterpenoid (chemical) family, was "strongly active" in the NCI anti-HIV screen, including HIV–2 and drug resistant HIV–1 strains (Gustafason et al. 1992). Samples of *H. nutans* were collected by Paul Cox because of its use in Samoan medicine that included the leaves for back pain and abdominal swelling, woody stem for yellow fever, and roots for diarrhea (Gustafason et al. 1992); other reports refer to its use against hepatitis. A question that arose was whether prostratin or other phorbol esters or tannins in the plant are responsible for the alleged Samoan claims? Through Paul Cox, the NCI obtained a sample (bark) of the preparation itself for evaluation, and found that prostratin was in such low concentration (37 µg/ liter) that it would be difficult to attribute it to the alleged claims, compared to the higher concentrations of other secondary metabolites in the preparation (Beutler et al. 1995), although the concentration of prostratin in *H. nutans* can vary (Johnson et al. 2008). In any case, two ethnobotanists were involved in the discovery of prostratin for its anti-HIV activity, while it seems that only Cox was recognized in a patent approved 26 Aug 1998 (EP19910910575). Ironically, Cox may have employed Uhe's (1974) *Medicinal Plants of Samoa* in the selection of his samples for the NCI screening.

However, the original medical parishioners in this same case might even be traced to earlier cultures in Morocco and China if other Euphorbiaceae are considered. The genus name *Euphorbia* was adopted by Linnaeus—internationally accepted as the starting point for botanical nomenclature—for Euphorbus, the name for the Greek physician, King Juba of Mauritania, 25 BC–23 AD, whose medicinal remedies included the dried milky latex of *E. resinifera* O. Berg & C. F. Schmidt, endemic to Morocco, a plant in which we (WBA) had received a request in 2003 from a pharmaceutical company to supply a large quantity of the milky latex for a clinical study of its antitumor properties. More than 70 of the ~2,000 species of *Euphorbia*, have been used against cancerous symptoms (Hartwell 1967–1971), and extracts from ~35 species were identified as active in the NCI screen (CPAM, 1977, 1982). Although it is usually the white sap in stems that is employed, the roots of *E. fisheriana* Steudel have been used in China against cancer for over 2,000 years (Mabberley 1997, not in Hartwell 1967–71). Roots of this species contain prostratin among other diterpenoids (Ma et al. 2007).

Species of *Euphorbia* (Euphorbiaceae), *Pimelea* (Thymelaeaceae), and many in other genera of their respective families, are well-known for their carcinogenic diterpenoid compounds that also inhibit tumor growth (Cashmore et al. 1976; Farnsworth et al. 1976; McCormick et al. 1976). However, the structurally related ingenol found in many species of *Euphorbia*, has been employed as a template for synthesizing anti-HIV triesters—such as ingenol-3, 5,20 triacetate—that are of interest because they are not tumor promoting. Thus, one might conclude that practitioners in many cultures have recognized the physiological effects produced by Euphorbiaceae and Thymelaeaceae plants, whereas **plant taxonomists**, chemists and pharmacologists are systematically identifying the chemical constituents that may be best employed in modern medicine; 28.5% of the extracts tested from plants of the Thymelaeaceae

had shown antitumor activity that included one species active in L-1210, and 14 others with T/C >175 in P-388, in contrast to 4.1% active extracts for 64,634 screened (Barclay & Perdue 1976). Additionally, recent discoveries in several other Euphorbiaceae appear promising, englerin A (*Phyllanthus engleri*) for treating colorectal cancer (Ratnayake et al. 2009) and schweinfurthins (*Macaranga schweinfurthii* Muell.Arg.) that may possibly demonstrate a novel mechanism of action, "especially glioblastoma lines SF-295 and SF-539" (Beutler et al. 2006).

Farnsworth and Soejarto (1991) extrapolated that 28% of the world's species may be used medicinally based on survey of 33,000 species; however, this estimate probably did not adjust for the widely distributed species that make up much of the folklore bulk. If Moerman's (2005) estimate of 8% is employed, then the world number of medicinal plants is approximately 20,000 species. The NCI history of random collections clearly shows that ethnobotanical species get collected; for example, Spjut (1985, USDA Memoranda: 1978) reported that only 5 of ~450 North American genera listed in Harwell's (1967–1971) 3,000 plants used against cancer had not been screened (*Ayenia, Gratiola, Limosella, Malaxis, Pinquicula*), compared to nearly 100 genera in just California alone that also had not yet been screened (Spjut USDA Memorandum 1979), and which for the most part are not known to be used in folk medicine.

Moreover, generating lists of plants used for specific medicinal remedies and then collecting them will not necessarily lead to a short-cut, or to cost effectiveness in making discoveries. Selective approaches to plant collections, whether medicinal or taxonomically based, is at least 10 times more expensive than a methodical random approach. A major problem is extracting meaningful data from literary reports and/or databases on medicinal plants in order to identify those species that are truly indigenous and have not already been extensively screened. The selected species then have to be evaluated in features of taxonomy, ecology, geography, abundance, plant parts, method of preparation, and whether it is feasible to obtain agreements and permits. Field explorations have to be planned in regard to length of time required with the idea of obtaining the samples in the shortest distance of travel, which might be compared to the "traveling salesman problem." In random taxonomic collections from temperate or Mediterranean regions, one may expect to collect 10–50 samples per day (Spjut 1982), or in tropical areas as many as 60 samples may be collected per day (Perdue & Hartwell 1969). In contrast, the selective collecting of plant samples—unless there are a lot of them—will likely be limited to one or two samples per day.

The Intellectual Property Rights to Plant Genetic Resources

The authors of various herbals who have published over the millennia have contributed their intellectual knowledge (IK) without expectation to royalty payments for any discoveries that may arise later. "Then in 1992 the Convention on Biological Diversity (CBD) declared exploitation of genetic resources to be the sovereign right of where the resources occur" (Lesser 1997). This is the year when taxol was approved by the FDA for treating ovarian cancer and when Merck gave the Instituto Nacional de Bioversidad in Cost Rica upfront payments of $1 million and $100,000 in equipment along with an undisclosed percentage of royalty payments for any discoveries arising from screening of Costa Rican plants. This is also when the WBA ran into difficulties in obtaining plant collecting permits for Western Australia.

Plant Collecting for Scientific Research in Western Australia (WA)

The Principle Investigator (PI) for the WBA obtained samples for cancer and AIDS research from Western Australia (WA) in 1981, 1986, 1990, 1991 and 1992. Species of smokebush (*Conospermum*, Proteaceae) were active in the anti-HIV screen based on samples the PI of WBA had collected in WA during Aug-Sep 1981 for anticancer screening. The NCI had developed its Letter Of Collection (LOC) in 1988 (Cragg & Newman 2005) for which we (WBA) had made known to our contacts in Australia, Mexico, and Ecuador. "The LOC states NCI's willingness to collaborate with local scientists and/or authorities in the discovery and development of novel drugs from organisms (plants, marine invertebrates, microbes) collected in their countries and/or territorial waters, and, if requested, the NCI will enter into formal agreements based on the LOC with the relevant source country government agency or organization" (Cragg & Newman 2005). There was interest in the LOC expressed by one key official in the WA Department of Conservation and Land Management (CALM) in 1991. However, he mentioned this in context of his private business in eastern Australia in having the capability to do tissue culture; thus, it was not clear whether this research, if needed, could be done privately or through CALM.

The original samples of WA plants were obtained in 1981 under a U. S. government cooperative research project between the NCI and USDA Agricultural Research Service (ARS) with the required permits from the WA government. In Oct 1981, the NCI natural products screening program lost its funding, and had to terminate its agreement with the ARS. At that time Spjut was in the mid point of collecting WA samples and had to immediately stop collecting. A concern arose as to whether the WA samples that had been collected should be shipped to the U.S. since the intended purposed of their collection could no longer be met. The curator of the WA herbarium at the CALM expressed his disappointment and no need for the samples to stay in WA. Spjut relayed these concerns to the ARS and to the Chief of the Natural Products Branch (Matthew Suffness) who then arranged to have the samples extracted at RALTECH and the extracts forwarded to three of their contract university chemists. Some of the extracts were screened in KB during the early 1980's and there was interest in doing follow-up recollections at RTI (see Wall et al. 1987).

The WA samples and their extracts at Purdue University—that had been tested in KB under the Chairman John Cassady—were retrieved by the manager of the NCI extraction facility in Frederick MD. The extracts of the WBA samples were then screened for anti-HIV activity. Several species were found to be of interest. In 1990 recollections were obtained through contacts recommended by CALM; however, in pursuing recollections, it was strongly indicated that the WA resources were limited, and that the WBA personnel should visit WA to obtain the recollections. In 1991, the WBA obtained recollections of several anti-HIV active species under a NCI/WBA Master Agreement Award with all the required permits from the WA authorities.

In 1992, however, CALM expressed delays in processing the WBA permits. One of the contacts who had assisted in the earlier collections indicated that follow-up recollections and survey of smokebush (*Conospermum* spp.) could be done under his permit. It was also suggested it would help alleviate concerns from the CALM by collecting under an Australian business; thus, WBA-Australia was established.

A key official in WA, who was not overly concerned about any agreement in 1991, became discontented with the LOC despite the fact that the WBA had established World Botanical Associates-Australia with strong legal support through its Australian partnership. They were also looking for upfront payments and the U.S. to finance development of their own screening program, and to finance their clinical trials of extracts from the plant (not the pure chemical, conocurvone). The WBA had made an investment for screening WA plants supported by the LOC with the intent to facilitate royalty payments for any discoveries, but as proprietary information was made available to WA through the permit process, the conditions for granting permits frequently changed, making it difficult to reach a mutual understanding and agreement. There were numerous lengthy discussions that took place in Perth—in person—between the WBA-Australia with its attorney present and top level officials in CALM. One of the issues we (WBA) were concerned about was CALM wanting to mask the identify of the samples in their code.

Fig. 4. *Various news articles on smokebush* (Conospermum incurvum) *published in 1992.*

Contact was also made with Kings Park for assessing their willingness to do research on *Conospermum* in tissue culture while one of the officials in CALM also had earlier expressed a similar interest (in 1991), but under a private company instead of the WA government as noted above. Thus, conflicting and competing interests were evolving. A business partner in the

WBA-Australia also had his revegetation business company and had formed another separate business enterprise with an attorney regarding the smokebush recollections and survey. The PI (Spjut) of WBA, then based in Laurel MD, had cautioned all WA contacts many times about the slim chances of a compound becoming a drug, especially when it had not even reached clinical trials, reminding them that it was still under isolation and evaluation.

A major issue to development of a new drug is its supply. The WBA mission in WA was to explore not only the active species but alternative species. The WBA had an agreement with CALM in 1992 for the collection of samples under the permit of its partner in WBA-Australia. Samples were also collected under permit from King's Park expert on the taxonomy of smokebush, a permit which also allowed for the collection of threatened and endangered species. The samples were left with the WBA-Australia in Perth, while the voucher specimens were taken away at the airport. In 1991, and as in 1990, and 1981, the modus operandi was to ship the vouchers separately to the U.S. The objections to taking the vouchers in 1992 concerned seed being present in the voucher that could be grown in U.S. labs; however, seed of *Conospermum incurvum* and other WA species were being sold by WA nurseries to those in U.S., Israel, and other countries for horticulture (Fig. 4).

Conospermum incurvum was collected based on taxonomy and novelty of the WA flora to the NCI screen as determined in the planning stages (Spjut travel report, 1982); see also NIH-NCI Memorandum from Matthew Suffness to James Duke (16 Jan 1981): "I was very impressed with Rich's workup on Western Australia, There is no doubt that this will be a productive area to collect in and I feel that this is a good example of what can be done to evaluate the potential of collection areas, select collection locales and collection times." It was not a plant known to have been used in traditional medicine; Spjut reviewed references in Australia on medicinal plants at the herbarium in Perth and in local bookstores. Spjut also had avoided collecting common genera such as *Eucalyptus* and *Acacia*; both have numerous species that if collected could have easily constituted the bulk of 758 samples in which case then the anti-HIV activity in smokebush may never have been discovered.

As indicated previously, taxonomy is an important tool for discovery of novel compounds in plants; however, a number of patents filed on the discovery of conocurvone and other compounds from *Conospermum incurvum* (Boyd et al. 1997, 1998, 1999) do not recognize one of the inventors. The taxonomy is clearly applied in the patents by reference to the scientific name, but not to the taxonomist (Richard Spjut) who collected the samples and identified them. Moreover, the taxonomist had been recognized by the former NCI Chief of the Natural Products Branch in the NCI, and by the Administrator of the ARS in regard to the planning and carrying out the collections of WA plants during 1981 (Spjut travel report 1982). "I am impressed with this fine example of evaluation technique that was applied by you." "Obviously your preparation was quite thorough and was rewarded with excellent results" (T. B. Kinney, Jr., Jun 16, 1982, Foreign Travel Report—Western Australia and Tasmania). "You really did a tremendous job from start to finish including organizing the trip, doing the advance work on selection of species to be collected, making the collections and doing the identifications and writing the report" (M. Suffness, May 11, 1982). Do not the terms "technique" and "selection of species" qualify as part of the basis for the invention? Another patent on the discovery of conocurvone was filed by

Stagliano (2004) with no mention as to the source of the discovery of the compound, the collector and its voucher specimen, (*Spjut & Edson 7139* NA, OSH, PERTH, US).

Plant Collecting for Scientific Research in Ecuador (Galapagos Islands)

In 1997, the WBA had reached a verbal agreement with the Director of the Galapagos Islands that would allow us to obtain samples of *Castela galapageia* Hook. f. for identifying the best source of quassinoid compounds in developing a semi-synthetic derivative for cancer chemotherapy. Paul Grieco, who had been working on semi-synthetic derivatives of quassinoids at Indiana University, had found it more economical to use the plant material rather than to create the semi-synthetic compound via total synthesis. However, one assistant under the Director of the Galapagos Islands National Park was not totally receptive to the agreement. A misunderstanding was the 1–2% royalty—in case the Ecuador species of *Castela* turned out to be a better source of the desired compounds than the ones we already had discovered from our earlier collections obtained in the United States and Mexico. He wanted 50%. We further explained that the royalty is based on gross sales, not net income, and that this could be a huge sum of money. While we seemed to have reached an agreement that allowed us to collect the samples under the direct observation of the Director and his assistant, we (WBA) never received the samples despite the verbal agreement followed by our signatures on a formal written agreement. Our case could have been strengthened if we had examples to show the authorities of known plant discoveries where remuneration (from royalties) was received, but we did not know of any; thus, the authorities seemed skeptical about getting their fair share (royalty) for any discovery that might result from chemical analysis of their *Castela* plants.

In contrast to the preceding case on smokebush, the WBA has been mentioned in a patent by Grieco et al. (2002, 2003). Paul Grieco had been referred to us (WBA) by Matthew Suffness in 1990 for finding natural occurring sources of chaparrin and chaparrinone. He specifically requested samples of *Castela nicholsonii* Hook., which we had determined to be a synonym of *C. tortuosa* Liebm. We subsequently supplied samples of this species from southern Mexico, and also of a closely related species, *C. texana* from Texas. We also recommended samples of other Simaroubaceae, based on antitumor activity in previously collections for the NCI, especially those that had not been previously recollected such as *C. peninsularis* Rose and *C. polyandra* Moran & Felger from Baja California, samples of which Spjut had originally collected during 1979 and 1980, both of which later led to discovery of novel compounds and their patents filed by Grieco et al. (2003). We also obtained samples of other species of *Castela* from South America and from the United States. The primary species of interest are those from Baja California. The lack of interest in *C. tortuosa* and *C. texana*, and also in *Holacantha*, suggests that samples of *C. galapageia* would not have been of interest since it has been included in a broader species concept under an earlier name, *C. tortuosa*.

Biochemical Screening of Natural Products for Big Business Only?

The NCI and the big pharmaceutical companies such as Merck, Bristol-Myer Squibb, and others, can afford to pay huge up-front fees and/or provide training, whereas universities and small companies may be left out (see also Barton 1997) unless they can collaborate with the big companies. Thus, plant collecting for biochemical screening has become a big business venture

with a minute chance for success. It has been estimated that about 1 in 10,000 species screened reach clinical trials and that the cost to develop a new drug from a plant source can exceed $300 million (Lesser 1997); a more recent cost estimate ranges from $800 million to $1.7 billion (Cragg & Newman 2005).

The law on intellectual property rights (IPR) allows for patenting of an identified active principle from a plant, but not for the plant or its folk uses; however, the indigenous people have a right to control the traditional knowledge (TK) of a plant's use, which therefore provides a foundation upon which an agreement may become necessary between a company and indigenous people's government (Barton 1994). This seems to have led to emphasis on traditional knowledge as a basis for agreements.

What if an investigator's discovery is decidedly based upon data from many cultures rather than an individual ethnic use? Should everyone receive royalties? From a broad point of view, it is *Homo sapiens* as a species that has found medicinal value in plants, not necessarily the TK of a particular culture. As stated by Lesser (1997): "Genetic resources were once treated as a common heritage available without restriction for research and other usage. The system was perceived as unfair to developing countries—the major source of genetic resources. Since the Biodiversity Convention declared that governments have the 'sovereign right to exploit' the genetic resources under their domain, efforts to regulate access have begun." So it would seem that 10,000 years have passed before man has decided that plants and their chemicals need protection from scientific studies for monetary reasons.

Although the source country where the plant sample was originally collected may be viewed as the one that should be compensated, this is not always clear-cut. For example, the alkaloids isolated from Madagascar periwinkle, *Catharanthus roseus*, were discovered from samples collected in India, Jamaica and the Philippines, which are outside the plant's native range (Summer 2000; Cragg & Newman 2005). Frisvold & Day-Rubenstein (2008) indicated that the people of Madagascar got left out of any return for the discovery because the samples came from somewhere else. There are many cultivated and naturalized plants whose native origins are not precisely known. This leads to other claims based on "breeder rights" and "farmers rights." Nonetheless, the emphasis on "traditional knowledge" appears to circumvent the "loophole" (Iwu 1997) in the CBD that allows for research on plants acquired before Dec 29, 1993. But as stated previously, "traditional knowledge" cannot be easily substantiated in view that it may have been acquired over time from knowledge that was dispersed from earlier Mediterranean cultures, dating back to ~10,000 BC.

Ethnobotanists who pursue medicinal folklore for new drugs deal largely with vascular plants (e.g., CIBA Foundation Symposium 1994); however, bryophytes (e.g., Spjut et al. 1986, 1988) fungi including lichens, marine organisms, microbial organisms, and animals in general do not fit into this folklore rationale. Many lichen and bryophyte species occur on many continents, because they generally arose before the split of Pangaea and because their rate of species evolution is much slower than higher plant groups.

What about migratory animals such as fish, mammals, birds, and butterflies that are not confined to a particular culture or country? For example, a monarch butterfly travels as much as

3,000 miles between Canada and southern Mexico (in four generations). Their larvae feed on milkweeds (*Asclepias* spp.) that have shown KB activity due to cardenolides (Hartwell 1976), which have also been found in butterflies; these compounds in milkweeds when consumed by butterflies help deter birds from eating them (Brower & Moffitt 1974). The "glassy tiger" butterfly, *Parantica aglea-melanoides* Moore, found in Kashmir to Myanmar, Thailand, Laos, Vietnam, Hainan, China, and Malaysia (http://yutaka.it-n.jp/dan/30080010.html) was active in KB based on a sample collected in Taiwan (CPAM 1977); it feeds on the milkweed *Tylophora carnosa* Wallich ex Wight. A related species, *T. crebiflora* S. T. Blake, contains the phenanthroindolizidine alkaloid tylocrebine, listed in Hartwell (1976) as one of the 21 most important anticancer compounds, and still is regarded as having potential for cancer chemotherapy (Ancuceanu & Istudor 2004).

Then there are endophytic organisms that have also been discovered in recent years to produce many of the antitumor drugs that have been isolated from higher plant tissues; examples have been found for camptothecin (Puri et al. 2005), podophyllotoxin (Eyeberger et al. 2006), the vinca alkaloids (Kharwar et al. 2008), taxol (Strobel et al. 2004), trichothecenes (Jarvis et al. 1981), triptolide (Kumar et al. 2005), and maytansinoids (Zhu et al. 2009). Endophytic fungi may cause the plant to produce the active compound for protection (Wilson 1993), or provide protection to the plant against insects or disease (Azevedo et al. 2000), or the plant may modify the endophyte's secondary metabolite as reported for *Baccharis megapotamica* Spreng. (Jarvis et al. 1981). This is an area of natural products that is virtually unexplored. Endophytic organisms also include flowering plants such as *Pilostyles thurberi* A. Gray (Rafflesiaceae), a sample of which was collected for the NCI by the WBA in 2002 (*Spjut & Marin 15122*, BRIT, WBA, US). Other associations may be more casual as evident between bryophytes and cyanobacteria (Spjut et al..1988), especially *Nostoc* in which species of *Nostoc* have yielded anti-HIV compounds (Boyd et al. 1997). As noted by Spjut et al. (1988), organisms in their natural environment are "more often than not symbiotic systems" (Lewis 1973).

What needs to be realized is that it is not the medicinal uses of plants in various cultures—linked to "traditional knowledge"—that should provide a basis for royalty rights, but the degree to which source countries conserve their natural resources. This would seem to have been the intent of the 1992 CBD. Country organizations that allow deforestation to continue out of control and then complain about not getting their fair share from any discovery seems hypocritical.

Many scientists and individuals—especially those who lack training in plant taxonomy—a field of study that draws on knowledge from many scientific disciplines (e.g., chemistry, cytology, ecology, geography, molecular, morphology, etc.)—have jumped on the bandwagon of advocating indigenous rights to plants based on "traditional medicine" even though the medicinal uses are unsubstantiated. This is like comparing the Food and Drug Administration (FDA) review of scientific evidence for approving a drug to that of the "alternative medicines" sold as dietary supplements for which the alleged benefits are questionable. It also seems that the CBD has led to expectations that novel drug discoveries are common occurrences from screening plants (Cragg & Newman 2005) and that all such discoveries must be rooted in traditional knowledge.

The real intellectual input in plant collections is to be found in the taxonomists who collect and identify the samples for biochemical screening. If it were not for the taxonomic identifications, the chemical structures would not have a nomenclatural reference to their origin. Authors in many pharmaceutical journals appear to give little attention to taxonomy (see also Wheeler 1997) as well as to the professional taxonomists who have acquired experience in applying plant taxonomic data. For instance, *Crossosoma bigelovii* (Crossosomataceae) was collected by Spjut with due respect to the unique taxonomic features of the genus and the need for additional samples based on its previous track record of its antitumor activity (http://www.worldbotanical.com/crossosoma.htm); yet, he was only acknowledged for his intellectual contribution (Klausmeyer et al. 2009) in contrast to other ethnobotanists who receive authorship by just talking to native practitioners.

The NCI has had cooperative agreements with 15 countries (Cragg & Newman 2005), and also Memoranda of Understanding with 24 countries, offering them secondary assistance in screening and training in the United States, essentially "technology transfer" (Cragg & Newman 2005). While this is beneficial to the research on natural products, it is U.S. tax dollars being utilized for creating jobs outside the United States when one might question as to whether these dollars should be equally or entirely directed to scientists in the U.S. "All results from such secondary testing are considered the sole intellectual property of the SCO (the NCI regards such testing as a routine service to the scientific community), and can be used by the SCO in the application for patents covering sufficiently promising inventions. The NCI will devote its resources to collaborating with the SCO in the preclinical and clinical development of any SCO-discovered drug which meets the NCI selection criteria, and will make a sincere effort to transfer any knowledge, expertise, and technology developed during such collaboration to the SCO, subject to the provision of mutually acceptable guarantees for the protection of intellectual property associated with any patented technology" (Cragg & Newman 2005). Nevertheless, Iwu (1997), had earlier suggested "a better approach is for developing countries to process their own traditional remedies as standardized drugs and seek to market them both within their own countries and in the international market."

These approaches and attitudes towards foreign investors may actually discourage research on natural products, which would also mean fewer discoveries. From a long term perspective, the more easily natural resources are made available to the worldwide research community, the more discoveries will likely come about.

Future Collection Strategies for Finding New Drugs from Plants?

Although the NCI has screened thousands of species since 1960, the screen has continued to evolve over the years as our understanding of cancer advances. There are many variables in the collecting and testing that may correlate for a given set of bioassays but not others. Fortunately, the NCI has established a repository of natural product extracts since 1985. It may be reasonable to conclude that the more widely distributed species such as those of pantemperate and pantropical distribution probably do not need further collecting. The main focus should be on subfamilies and genera of plants that have not been previously collected, and in geographical areas where collections have already been made, additional collections should consider root, bark and fruit samples that have not been previously collected.

The WBA has focused on collecting in desert communities in the United States and its Territories since 1985. Unlike vegetation in other Mediterranean climates, the floras in the western US notably differ in the large diversity of annuals. The weight requirements for dried samples (500-2000 g) have limited their collection, however. As noted earlier ~ 100 genera native to California (excluding grasses) have never been collected for the NCI. A major problem besides weight requirements is over-grazing and Off-Road-Vehicle recreation, especially since the year 2000 (Spjut submitted). These activities appear related to increasing abundance and range expansion of invasive species, while native herbaceous species appear in decline. Other limitations to collecting samples for the NCI are due to the expansion of national parks and wilderness areas that leave less territory for collecting outside these areas. In 1972 samples were supplied to the NCI at the cost of $5.00 per sample; in 1978 it was ~$30 per sample (Spjut 1985); in 2001, it was ~$50 per sample; and in 2008, it was nearly $200 per sample.

While there has been an emphasis by some investigators to target plants based on use in traditional medicine, these investigations have their own merit in the documentation of our cultural heritage. Unless investigators pursue the remedies according to the plant species, parts, and preparation methods, the taxonomic method to sampling remains as the most effective method to discover new leads.

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