Taxus baccata L.
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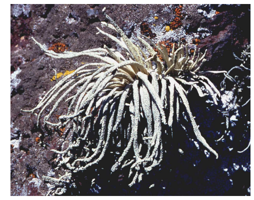

Niebla tesselata Spjut
holotype

Final Report: NCI Plant Collections and Taxonomy
United States and Territories
N02-SC-17013-30
Sep 1, 2001–Aug 30, 2004

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Objectives

To supply the National Cancer Institute (NCI) annually with 1,000 novel plant samples represented by a broad diversity of taxa from the United States (US) and Territories in screening for new anticancer compounds for a 3–5 year period. Samples of selected plant parts, or the entire plant, are collected in quantities to yield 300–500 g dry weight for extraction and testing against 60 different cell lines of cancer. The geographic areas for collections are determined by phytogeographical relationships between species previously collected within the United States (Morton Arboretum, 1997–2001) and those not yet collected. Collections are carried out in accordance with the NCI work statement and funds allocated.

Summary

The World Botanical Associates (WBA) analysis—in response to the NCI RFP—revealed that most samples collected in the US were from the eastern deciduous forests and the northern coniferous forests (Morton Arboretum, 1997–2001, 5,002 samples).

The WBA supplied the NCI with 2,744 samples primarily from the Southwest (including southwestern Texas), Alaska, Hawaii and Puerto Rico. These represented 1,150 species and varieties in 621 genera and 156 families (Appendix I). Genera new to the NCI screen were mostly from California. The 1,000 sample quota was met for the first two years, but in this (3rd) year, 744 samples were provided due to 10% budget cuts in the preceding years followed by notice in March 2004 of termination of plant collection contracts; the WBA had planned to reduce collections in the 5th year according to the budget. The WBA spent most of this year's funds on collecting 671 samples in

higher cost areas—Hawaii (Sep-Oct 2003) and Puerto Rico (Feb. 2004)—that were scheduled for years IV and V with 30% more funds. Another 300 samples were planned for the spring and summer from the national forests of the Californian Region, Pacific Northwest Region, Rocky Mountains Region, and Intermountain Region. Permits were received from all major forest regions, and most forest districts in California. Only 73 samples were collected from BLM and private lands in California and Nevada in March (2004). None were collected from the forest regions because of the unexpected short notice for termination of the contract. Samples from the US mainland include 24 fresh samples from 24 species.

In this final report, the WBA systematic approach to plant collections is discussed based upon what has been collected not only since the current phase of the NCI program—1986 to present, but also since the program's inception—1960. The WBA develops a strong justification as to why its samples are most likely to yield novel compounds based on a review of past collections for the NCI screen, by emphasizing that the more frequently active plant parts—root, bark, and flower-fruit—have not been provided to the NCI for many genera and species until the present contract with the WBA.

A Review of Plant Samples obtained from the United States for the NCI Antitumor Screen

The NCI has screened plant extracts under two long-term programs, (1) ~120,000 extracts from ~35,000 plant **species** during 1960–1982 (Spjut 1985[1]), and (2) more than 100,000 extracts since 1986.[2] The Agricultural Research Service was the major supplier of samples from the US and foreign areas for the initial 22 years (1960–1982). Other suppliers were the University of Arizona who collected and extracted ~3,500 general samples from Arizona and Sonora (Mexico) mostly in the 1960's, the University of Hawaii who collected an undetermined number of samples in Hawaii during the 1970's, and George Petit at Arizona State University who collected, for example, only "pl" samples from ~ 200 species of shrubs and herbs in Arizona, California, Oregon, and Washington during 1975. The Hawaiian samples, which were screened against KB and PS, were reported on earlier in regard to recent WBA collections from Hawaii. Therefore, other US collections will now be reviewed.

The NCI antitumor screening has identified active extracts from more than 3,000 plant species; however, it appears that less than 25 have undergone clinical studies, and only two so far have yielded natural compounds or their derivatives that are used commercially in drugs for treating cancer, camptothecin from *Camptotheca acuminata* (Nyssaceae), native to southern China, and taxol from *Taxus brevifolia* (Taxaceae), a gymnosperm native to the Pacific Northwest. Additionally, novel anticancer drugs have

[1] Spjut, R. W., 1985. *Limitations of a Random Screen: Search for New Anticancer Drugs in Higher Plants,* Economic Botany 39: 266-288.
[2] Cragg, G.M., J.E. Simon, J.G. Jato, and K.M Snader. 1996. *Drug discovery and development at the National Cancer Institute: Potential for new pharmaceutical crops.* p. 554-560. In: J. Janick (ed.), Progress in new crops, ASHS Press, Arlington, VA. The authors had reported 87,000 extracts.

been discovered from investigations of chemical compounds in two other plants, the vinca alkaloids from the Madagascan periwinkle, *Catharanthus roseus* (Apocynaceae), and podophyllotoxin from the eastern US may-apple, *Podophyllum peltatum* (Berberidaceae).

Plants Collected by the University of Arizona for Antitumor Screening

The University of Arizona collected and extracted both fresh and dried samples of vascular plants; ~5,067 of their extracts from 3,500 samples were screened by the NCI. An analysis of 1,044 species of angiosperm collected between 1960 and 1964, which had excluded the genus *Agave*, revealed that most samples were of the entire plant (pl), or of the aerial parts without root (px).[3] Occasionally, root (rt)—55 samples, stem-bark (sb)—20 samples, and other parts were collected. Two extracts (aqueous and alcohol) were prepared from each sample.[4] Nearly all were tested against SA-180 and LE-1210. About half were screened against KB and other tumors (D1, FV, WA, LL, CA).[5] The incidence of activity in one or more tumors was slightly higher in fresh samples than dried samples—2.6% in aqueous extracts of fresh samples compared to 0.8% in ethanol-chloroform extracts of dried samples; however, there was a notable difference for one tumor—KB; there were 28 active KB extracts from fresh samples (11 aqueous, 17 alcohol) compared to just one KB active from dried samples. Later collections were mostly dried samples from which only one aqueous-ethanol extract was prepared. These again were mostly aerial parts (pl, px)—from ~570 angiosperm species; root samples were collected from 15 species and stem-bark from just one species.

In a strict comparison of plant parts, regardless whether collected fresh or dry, or whether from an aqueous or alcohol extract, the incidence of activity was highest in stem-bark—11.1%, followed by root—4.0%, and then aerial parts (px, pl)—2.6%.

Active agents included betulin (WA, FV, D1) from fresh *Alnus firmifolia* sb (Mexico), sequiterpene—paucin (KB, PS 138%) from dried *Baileya pauciradiata* pl, "protein like" (FV) from fresh *Caesalpinia cacalaco* st-lf-fl, protein—cesalin (SA) from fresh *Caesalpinia gilliesii* fr,[6] sequiterpenes—arctiopicrine (KB, PS 140%) and artemisiifolin (KB, PS 130%) from fresh *Centaurea melitensis* pl, "protein" (LL, WA) from dried *Cercidium microphyllum* rt (AZ), steroid lactone—withaferin (KB) and 5,7 dihydroxy-8-

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[3] Data were compiled by Richard Spjut during 1978–1979 as an employee of the ARS to evaluate activity according to plant parts and vegetation types, and to determine which genera were only collected by the University of Arizona. Matthew Suffness provided Spjut with a computer printout of all the University of Arizona collections (Code 315E). Conifers and *Agave* were excluded because it was apparent that efforts were made to collect samples of these taxa due to antitumor activity of interest in *Taxus, Cephalotaxus*, and *Agave.*

[4] For 300 or more samples, there were three types of extracts, an aqueous prepared from boiling water filtered through a cotton-gauze, evaporated and lyophilized, another aqueous prepared by stirring and filtering through glass wool with the filtrate reduced to a dry powder by a Stokes Freeze Drier, and a third with ethanol-alcohol (95%) and chloroform, see below footnote no. 5 for reference.

[5] Screening Data from the CCNSCSL, 1966. XXXIV. Plant Extracts. Abbott, BJ., J. Lotter, J.L. Hartwell, M.E. Caldwell, J.L. Beal, R.E. Perdue, Jr., & S. A. Schepartz. Cancer Res. 26 (7), pt. 2: 761–935.

[6] Doskotch at Ohio State University reported a bisbenzylisoquinoline alkaloid, cosculine (KB), from sd of *Caesalpinia gilliesii* (Hartwell, 1976).

methoxy-2-methylchromone (KB) from dried *Crossosoma* "*parviflorum*" px (AZ)[7], cucurbitacin B (KB) from fresh *Cucurbita digitata* rt, "protein" (LL, SA, WA) from fresh *Mirabilis multiflora* rt,[8] "protein" (SA) from fresh *Osteomeles schwerinae* st-lf-fr, "phytosterol" (FV) from dried *Populus fremontii* sb, "tannin" (SA) from fresh *Rumex hymenosepalus* tuber, betulinic acid (SA), ursolic acid (PS 125%), and uvaol (PS 125%) from dried *Vauquelinia corymbosa* st-lf-fl, and sesquiterpene—zaluzanin (PS in vitro only) from dried *Zalulania parthenioides* pl. Active species from aqueous-ethanol extracts of dried samples included *Bouvardia ternifolia* pl (protein—bouvardin, WA), *Peucephyllum schottii* tw-lf (PS marginal), *Physalis crassifolia* pl (KB), and *Polygala macradenia* (PS).

Plant Samples Procured by the USDA Agricultural Research Service (ARS)

Most collections obtained by the ARS for the NCI—during the early 1960's—were from North America, which included many samples from Mexico. Most collectors were employees of the ARS; they involved the Principal Investigator, Robert Perdue, and other botanists, namely Edward Terrell (recollections, US wide), Arthur Barclay (general samples, Western US), Lloyd Spetzman (general samples and recollections, PR, AK, MN, MO, MI, NM, WI, NC, UT), Clyde Reed, (occasional samples, VA, MD, mostly general via contracts), Roger Miller (general samples, CA), Howard Gentry (mostly outside US, TX, general samples), and Cecil Smith (recollections, NC, VA) under Branch Chiefs Carl Erlanson, John Creech, and Quentin Jones.[9] Their field work was usually defined by political (state) boundaries, in contrast to the WBA phytogeographic approach. Fresh samples were also collected for the NCI by Perdue from the southern Appalachian states.

By 1969, the ARS botanists had collected 19,000 samples; the first 10,000 were represented by 5,478 species in which 11% were confirmed active in one or more tumors (Perdue and Hartwell 1969).[10] Many actives in SA, WA and LL were due to ubiquitous tannins and phytosterols;[11] nevertheless, a broad spectrum of other novel biological active

[7] Cole seemed to be interested only in *Crossosoma* samples from near Kingman, AZ that they identified as *C. parviflorum* based on Kearney & Peebles *Arizona Flora*. Samples collected elsewhere from Arizona and California were not "consistent" in "testing" (Spjut phone comm. with Wiedhoff, Sep 26, 1972, noted on "active sheet"). It is interesting that samples of *C. bigelovii* obtained from the Vizcaíno Desert of Baja California, screened 20 years later by Ching-jer Chang at Purdue University, had similar problems in reconfirmation of activity, but in this case the recollection was obtained at the same location as the original sample. It may be that active agents of *Crossosoma* react upon fractionation; thus, activity becomes lost.

[8] Novel antiviral proteins have also been isolated from fresh root of related species. J M. Vivanco, B J. Savary, & H. E. Flores. 1999. *Characterization of Two Novel Type I Ribosome-Inactivating Proteins from the Storage Roots of the Andean Crop Mirabilis expansa*. Plant Physiol. 1999 April; 119 (4): 1447–1456.

[9] List of USDA Plant Explorers and their explorations since 1898, compiled by R. W. Spjut, dated Sep. 27, 1985.

[10] Perdue, R. E., Jr. & J. L. Hartwell. 1969. *The Search for Plant Sources of Anticancer Drugs*, Morris Arb. Bull. 20: 35–53.

[11] Hartwell J. 1976. *Types of Anticancer Agents Isolated from Plants*, Cancer Treat. Rep. 60: 1031–1067, reported that activity in 164 species was due to tannins, and activity in another 62 species was due to phytosterols. .

agents were discovered such as iridoid lactones (e.g., penstemide from *Penstemon deustus*, KB, PS 184%), sesquiterpene lactones (e.g., eriofertopin from *Eriophyllum confertiflorum*, PS 148–167%, LE, B16), diterpenes (daphane type from *Dirca occidentalis*, PS, jatrophone and triptonide from *Jatropha gossypifolia* PS 145%), ansa-macrolids (e.g., colubrinol from *Colubrina californica*, *C. texensis*, KB, PS 154–213%), quassinoids (e.g., holocanthone from *Holocantha emoryi* PS 227%, B16), cucurbitacins (e.g., *Brandegea bigelovii* KB), triterpenes (e.g., uvaol from *Bursera microphylla*, PS 125%), lignans (e.g., deoxypodophyllotoxin from *Bursera microphylla*, LE, PS 154%, KB), more than 75 different alkaloids (e.g., pilocerine from *Lophocereus schottii* KB, senecionine from *Senecio* spp.), and miscellaneous other compounds. [12]

Additional samples from the US were obtained between 1970 and 1974 by subcontracts, Spjut in California (until May 1972), Johnnie Freeland in Louisiana and Arkansas, and Elkins in Texas. Spjut, during the spring and summer of 1971, collected in northern California 100 new samples not previously collected by Barclay based on a list of species he received from the USDA of Barclay's collections from California, Nevada, Oregon and Washington. Late in 1971, he collected samples from several hundred species in southern California without having to avoid species previously collected—for screening against the P-388 (PS) tumor that had recently become a routine assay, and also larger samples of active species (e.g., *Asclepias albicans, Baileya pauciradiata, Crossosoma bigelovii*), "FOSI" (Families of Special Interest, Apoocynaceae, Celastraceae, Rutaceae) and other "POSI" (Plants of Special Interest, e.g. *Colubrina*) for isolation of active agents.

From 1972 until May 1978, the ARS Medicinal Plant Resources Lab obtained general samples and recollections largely by contracts with suppliers in foreign areas; the general samples were screened against KB and P-388. Spjut, as an employee of the Medicinal Plant Resources Lab., reviewed taxonomic, ecological and geographical data on more than 200 antitumor active species to determine where they could be collected by subcontractors. During the six year period, field work by ARS botanists was undertaken primarily for massive samples that required 1.5 tons of *Colubrina californica* ws-sb-tw-lf and *C. texensis* ws-sb-tw-lf, 4 tons of *Thalictrum dasycarpum* fruit, 15 tons of *Maytenus buchananii* ws-sb, 5 tons of *Bouvardia ternifolia* pl, 5 tons of *Baccharis megapotamica* pl, and many tons of *Taxus brevifolia* sb for preclinical evaluation and clinical trials of active compounds. Botanists of the ARS New Crops Research Branch had earlier made small recollections of *Holacantha emoryi* from Arizona and California (Spetzman, Terrell, and Barclay, 1967–1969) and a large recollection of *Acer negundo* from

[12] Hartwell J. 1976. *Types of Anticancer Agents Isolated from Plants*, Cancer Treat. Rep. 60: 1031–1067. PS% is the increase in life span, test/control, in leukemic mice, data from Hartwell, Cole's progress reports, and references cited below:
Douros J & M. Suffness, 1978. *New Natural Products of Interest under Development at the National Cancer Institute*. Cancer Chemother. Pharm.acol. 1:91–100.
Suffness M. & J. Douros, 1979. *Drugs of Plant Origin*, Methods of Cancer Research 26:73–125.
Cassady J. & M. Suffness, 1980. *Terpenoid Antitumor Agents*. In J. Cassady & J. Douros, ed., Anticancer Agents Based on Natural Product Models, p. 201–269, Academic Press, NY.
Douros J. & M. Suffness, 1981. *New antitumor substances of natural origin*. Cancer Treat. Rev. 8: 63–87

Wisconsin (Spetzman in 1971). There were also smaller recollections (50–250 kg samples) of many species from East Africa, Arizona, and California, obtained by Spjut for isolation of active compounds, and there were cultivation projects on promising anticancer plants such as those mentioned above (except *Taxus*), and other species (e.g., *Brucea antidysenterica, Cepahlotaxus harringtonii, Putterlickia verrucosa, Tripterygium wilfordii*, *Heliotropium indicum*), and there were reviews on collection and screening data for a symposium held by the Society for Economic Botany on *Plants and Cancer* in Baltimore MD, June 15–18, 1975, published in Cancer Treatment Reports, Vol. 60, 1976. Examples of US plants recollected by Spjut were: *Amsonia hirtella, Arnica chamissonis var. incana, Asclepas albicans, Astragalus lentiginosus, Brandegea bigelovii, Brickellia atractyloides, Calystegia soldanella, Crossosoma "parviflorum," Croton californicus, Datisca glomerata, Dirca occidentalis, Eriastrum diffusum, E. wilcoxii, Erioneuron pulchellum, Eriophyllum confertiflorum, Eriophyllum lanatum, Erysimum capitatum, Ipomopsis aggregata, Iris missouriensis, Gutierrezia microcephala, Heliotropium curassavicum, Horkelia fusca, Palafoxia arida, Penstemon deustus, Kalanchoe tubiflora, Paxistima myrsinites, Physalis crassifolia*, *Pleurocoronis pluriseta, Sidalcea oregona, and Veratrum californicum*.

In 1976, the NCI modified its extraction procedure and focused recollections on species with activity in P388 >150%.[13] The new extraction procedure for general samples required 3–5 pounds instead of 1 pound (dry weight). In response to the NCI request to accelerate the development of active compounds to clinical studies (Chief John Douros), Perdue recommended that the chemists be supplied with the all the material they need in the initial recollection for isolation and preclinical studies since collectors can often obtain additional amounts above 50 pounds with less additional effort than the first 50 pounds, and that the overall cost per pound would be less.[14] Subsequent recollections increased from 50 pounds (Perdue & Hartwell 1969, l.c.) to 300 pounds for plants assigned medium priority (T/C 150–175%) and to 500 pounds for plants assigned high priority (T/C >175%); a 1980 ARS budget had planned to procure 15 high-priority plants and 35 medium-priority plants.

In May 1978, Jim Duke became leader of the Medicinal Plant Resources Lab with A. S. Barclay as Principal Investigator of the plant collections for the NCI, until his illness in 1980.[15] Under this new leadership, the NCI requested that lab botanists devote more time

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[13] Suffness, M., D. Statz, E. Wonilowicz & R. Spjut. 1988. *The Utility of P388 Leukemia compared to B16 Melanoma and Colon Carcinoma 38 for in vivo Screening of Plant Extracts*. Phytotherpy Res. 2(2): 89–97. It was concluded that extracts with marginal activity in P388, based on a current study of 99 plant species from a diversity of genera and families, would not show activity in solid tumors, B16 and Colon 38.

[14] Spjut did not concur with this approach because populations of species that may yield 50 pounds of dried material may not yield more.

[15] Hartwell had recently retired. His replacement, John Douros, previously with the fermentation program, was less familiar with all the intricacies of cross-linked records and codes in the botanical program that had been developed by Hartwell and Perdue over the past 15 years. This may have been frustrating to Perdue. Within the Medicinal Plant Resources Lab (MPRL, later changed to Economic Botany Lab), it generally took 3 years for one to become familiar with the program records dealing with the MPRL data and the NCI data. Moreover, it seemed that only Spetzman and Spjut had learned the program files, but Spetzman retired in 1974. Neither Duke nor Barclay appeared to take the time to learn the data

to field work, especially recollections, but also to collecting general samples, including the US. Spjut and Sandra Saufferer independently looked at what species might still be collected in the US, in addition to foreign areas. Gudrun Christienson, for example, collected in the Amazon of Venezuela, Jim Duke traveled to China, Egypt, Ecuador, Panama, and other areas during 1979-1980, and Spjut collected in the US, Mexico and Australia during 1978–1981. Saufferer obtained recollections and general samples from New Mexico and Puerto Rico (1979–1980). Also, Spjut, Saufferer and Christienson traveled together on one trip to Virginia, North Carolina and Tennessee in 1978 to obtain general samples of plants rarely screened by the NCI, and Duke collected more samples in North Carolina during May–June 1981.

Spjut further reviewed plants used by the American Indians of Nevada and elsewhere in western US. Although many plants reportedly used in American Indian medicine had already been screened by the NCI, the specified plant part often had not been collected, especially root and bark. Spjut, in a USDA Memorandum (Mar 28, 1978; Exhibit VI in RFP), reported that for 44 species of medicinal plants under consideration for field work, only 34% had been collected according to the specified plant parts in which root (rt including bu), stem-bark (sb) and fruit (fr) represented 63% of all parts reported.[16]

It might be noted that Perdue et al. (1970)[17] had concluded that—at least for herbaceous species—there was only a slight advantage to making a separate sample of root, in comparison to samples of the entire plant, or above ground parts; nevertheless, this study did not include shrubs or trees, perhaps, because collectors did not collect root from woody angiosperms as further detailed in the following examples.

> In a review of 179 samples of angiosperm species collected by R. Miller from California during Mar–Apr 1965 (PR 9747–9926), there were 3 root, 13 stem-

organization within the MPRL/NCI program. Perdue's program assistant, Mae Hatcher, comprehended the filing system, but lacked botanical training. Additionally, Goodman and Walsh in *The Story of Taxol* (2001) discuss the "Demise of the NCI-USDA Plant Screening Programme" in which they noted that Perdue had felt that there should have been more KB actives. In a retrospective study he conducted later (after May 1978), he found evidence of a sharp decline in KB actives in 1964 and suggested that this could be related to contamination of the KB with HeLa cells. On the other hand, there is only a finite number of KB active plants that exist out there, and that the discovery of new KB actives has to taper off at some point; perhaps, it was in 1964—at least for US species where the NCI had screened ~6,000 of the ARS samples by 1964. Once a species was reported active, the NCI-USDA would exclude a later collection of that species from the screen—to avoid duplication. Also, Hartwell did a statistical study that determined species with negative results in 10 or more extracts were less likely to show activity in future collections. Similarly, Spjut (1985, l.c.) reported that the chances of finding activity in a genus decreased after seven species had been screened without success. A point of diminishing returns in finding novel leads from a biodiversity ("random") approach depends on the sampling strategy as well as the bioassays and extraction procedures. Perdue's graph of %KB actives over time (unpublished) also showed an increase in 1970. This may be the result of more thorough sampling of East African plants by Perdue during the late 1960's.

[16] Plants were selected based on alleged uses in having a strong therapeutic effect, or were intensely sought after by the American Indians (e.g., *Enceliopsis*); see also Spjut, R. W. & R. E. Perdue Jr., *Plant Folklore, A Tool for Predicting Sources of Antitumor Activity?* Cancer Treat. Rep. 60: 979–985.

[17] Perdue, R.E., B.J. Abbott & J.L. Hartwell, 1970. *Screening plants for antitumor activity II. A comparison of two methods of sampling herbaceous plants.* Lloydia 33: 1–6.

bark and 7 flower-fruit samples. His samples from shrubs, for instance, *Adenostoma fasciciulatum, Dendromecon rigida, Acacia longifolia, Ribes sanguineum, Myrica californica, Ceanothus griseus*, and others, were represented by only twig-leaf. Occasionally, Miller collected an additional "older stem" (ws-sb) sample from species such as *Sambucus caerulea, Rhus integrifolia, Ephedra californica, Aesculus californica*, and *Cornus glabrata*, but no root. On the other hand, he collected stem-bark from 14 other species.[18] The three root samples were from herbaceous species, *Limonium californicum, Paeonia brownii,* and *Marah fabaceus.*

Barclay also collected in California during March 1965, 223 samples from 164 angiosperm species (PR 9519–9742). He collected relatively few samples of root (four) and stem-bark (five). As with Miller, Barclay's root samples were only from herbs—*Amsinckia tesellata, Astragalus parishii, Dicentra chrysantha*, and *Rumex hymenosespalus*, while his shrub samples were represented by just twig-leaf as exemplified by *Ribes indecorum, Arctostaphylos sp., Ceanothus tomentosus, Baccharis viminea, Rhus integrifolia, Rhamus crocea, Leptodacyton californicum, Ceanothus spinosus, Simmondsia chinensis, Rhus ovata, Isomeris arborea*, and many others.

However, Barclay in a previous trip to the Pacific NW, June–August 1962, had collected more root and bark samples that included, fortunately, *Taxus brevifolia,* the bark of which is not easily collected. Among 478 samples of angiosperms collected by Barclay (PR 4851–5000, 5066–5253, 6236–6334, 6912–6948), there were 56 root, 26 stem-bark, and 18 flower-fruit samples. His root samples from shrubs and trees—that were not collected again—until the NCI contract with the Morton Arboretum—were from such plants as *Populus trichocarpa, Rhamnus purshiana, Prunus emarginata, Castanopsis sempervirens, Symphoricarpos mollis, Spiraea densiflora*, and others.[19]

Otherwise, the USDA-ARS accession records clearly show a pattern that root samples were usually collected from herbs, while shrub samples were limited to just twig-leaf, except for an occasional woody-stem sample (ws-sb) as described for collections by Miller and Barclay, and also further exemplified in Perdue's collections from New Mexico and Utah during June–July, 1964 (PR-8408–8517, 8524–8661). Among the 248 samples Perdue collected from angiosperms, were 31 root, 6 stem-bark, and 18 flower-fruit samples. All root samples were from herbs, while shrub species such as *Rosa woodsii, Ribes aureaum, Atriplex canescens, Chrysopsis villosa, Rhus trilobata*, and others were represented by only twig-leaf, except for two species, *Ptelea trifoliata* and *Ceanothus velutinus*, that also included ws-sb (woody-stem with bark).

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[18] Stem-bark (sb) samples were obtained from conifers, which are excluded here, and occasionally from angiosperm species of oak (*Quercus*), cottonwood (*Populus*), ash (*Fraxinus*), and willow (*Salix*).

[19] Barclay on this trip was assisted by several students; in later trips he worked alone. Spjut, R. W. 1985. USDA explorers and their areas of exploration since 1898 (, compiled by Spjut, Sep. 27, 1985, Plant Taxonomy and Exploration Lab.

However, Perdue changed his sampling methodology in East Africa where he invariably collected root and bark samples from shrubs and trees. The lower cost in labor no doubt was a factor (Perdue & Hartwell 1969, l.c.). The significance of active agents being concentrated in root and bark is clearly evident from his collections and others from Africa, Turkey, and the Amazon in Peru as reported in previous reports for this project and on the WBA website (www.worldbotanical.com).[20]

Beginning in May 1978, Spjut made a special effort to collect root samples of desert shrubs and perennials, but he also had to avoid collection of species based on taxonomic guidelines that had evolved (Spjut 1985, l.c.). His work was further supplemented by Barbara Ertter and Jeff Strachan who collected in California, Nevada, and Idaho during 1980–1981 under contract with the Economic Botany Lab. They obtained a list of the plants that Spjut had collected prior to 1979, and then proceeded to collect samples of species and genera new to the NCI screen.

In addition to vascular plants, Spjut initiated the collection of bryophyte samples in 1979, although few bryophyte samples had been obtained by Clyde Reed in the eastern US and by Lloyd Spetzman in Alaska. These data were published in Economic Botany (Spjut et al., 1986, 1988).[21]

During 1983–1992, the WBA also continued collecting general samples and recollections of bryophytes for antitumor screening, mostly for chemists affiliated with the NCI program, Professor David G. I. Kingston at Virginia Polytechnic Institute and State University, and Dean John Cassady at the School of Pharmacy, Ohio State University. Screening data on these collections were summarized in Spjut et al. (1992).[22] Active agents included novel compounds such as ohioensins from *Polytrichum ohioense*, pallidisetums from *Polytrichum pallidisetum*, known ansa-macrolids from *Claopodium crispifolium* and *Anomodon* spp. based on initial activity in KB and P-388 assays, and novel sesquiterpenes, rivulactone and others from *Chiloscyphus rivularis* that were isolated based on activity against human lung carinocoma and a yeast-based DNA-damaging assay.[23] Collections of vascular plant samples were also made by Spjut from much of the US for antitumor screening by Professor Ching-jer Chang at Purdue University; many samples at Purdue University have since been retrieved by Thomas McCloud for inclusion in the NCI natural products repository of extracts for future screening by the NCI.

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[20] M. Suffness and J. Douros, in *Drugs of Plant Origin*, Methods of Cancer Research 26:73–125, 1979, stated that active agents are concentrated in root, bark and fruits but do not provide a reference for this.

[21] Spjut, R.W., M. Suffness, G. M. Cragg, and D. H. Norris. 1986. *Mosses, liverworts and hornworts screened for antitumor agents*. Economic Botany 40: 310-338.
Spjut, R.W., J. M. Cassady, T. McCloud, D.H. Norris, M. Suffness, G.M. Cragg, and C.F. Edson. 1988. *Variation in cytotoxicity and antitumor activity among samples of a moss, Claopodium crispifolium (Hook.) Ren. & Card. (Thuidiaceae)*. Economic Botany 42(1): 62-72.

[22] Spjut**,** R. W**.,** D. G. I. Kingston and J. M. Cassady. 1992. *Systematic Screening of Bryophytes for Antitumor Agents*. Tropical Bryology 6: 193-202.

[23] Chongming, W., A. A. Leslie Gunatilaka, F. L. McCabe, R. K. Johnson, R. W. Spjut, & D. G. I. Kingston. 1997. *Bioactive and other sesquiterpenes from Chiloscyphs rivularis*. J. Nat. Prod. 60 (12): 1281-1286.

In 1984, Spjut formulated a lichen collection project for which the WBA collected lichens from most of the US and Baja California (Mexico) during 1985 and 1986. Lichens were initially screened for anti-HIV activity by the NCI, but also for antitumor activity by Kingston based on collections Spjut obtained from the Inter-Mountain Region in 1984; the 1984 samples were identified by Mason Hale at the US National Herbarium, whereas Spjut identified the later samples, except Parmeliaceae identified by Hale. Recollections of many lichens were made for the NCI during the late 1980's and early 1990's. Anti-HIV activity in many lichens was reportedly due to polysaccharides. Hartwell (1976, l.c.) also listed usnic acid as an active antitumor substance, while lichens containing norstictic acid have shown activity of interest in the NCI screen (McCloud, pers. comm.). Both acid compounds are widely distributed in lichens.

In 1986, the NCI, after four years from termination of its previous contract with the ARS, began another era of sample acquisition for antitumor screening. For the first ten years, the NCI obtained samples mostly from tropical areas, ~50,000 samples supplied by the Missouri Botanical Garden, the New York Botanical Garden and the University of Illinois at Chicago with assistance from the Arnold Arboretum and the Bishop Museum in Honolulu (Cragg et al. 1996, l.c.).

In September 1996, the NCI decided to make an intense effort to screen samples of vascular plants from the US. The first 5,000 samples were supplied by the Morton Arboretum. In Sep 2001, the WBA was awarded the 3–5 yr contract, in response to a competitive RFP (Nov. 2000). The WBA proposed that collecting would be most productive in the southwestern US.

The WBA Collections for the NCI, 2001–2004

The WBA collected 2,744 samples from 621 genera and 1,180 species and varieties (Appendix I). Most were from California, Texas, Nevada, Alaska, Hawaii and Puerto Rico. Smaller numbers were obtained from Washington, Oregon, Arizona and New Mexico; see previous annual reports for details on numbers from each state, and reports on Puerto Rico and Hawaii for details on collections from those areas. The WBA samples from Hawaii and Puerto Rico were largely from woody plants, while those from the mainland included many herbaceous species. The combined total for both the MA and the WBA is ~7,746 samples collected from the US since 1997.

Due to 10% budget cuts and collecting in higher cost areas, the 744 samples for this year are still in accordance with the contract terms as recognized by the Project Officer. The WBA collected in the higher cost areas—Hawaii and Puerto Rico—to comply with the NCI request that collections be obtained from such areas in the first three years.

It should be noted that ~31% of the MA samples (5,002) belong to ten taxonomic groups of arborescent plants as shown in the following table. This in contrast to 5% being represented in the total WBA samples (2,744). Gymnosperms, as might be expected, represent a significant portion of the MA samples, 536 samples compared to just 39 samples collected by the WBA. Many gymnosperm species collected by the WBA were limited to samples of just fruit (cone).

Taxonomic group	**MA** Samples	**WBA** Samples
Gymnosperms	536	39
Quercus	307	12
Betulaceae	145	16
Salix	135	35
Acer	117	0
Prunus	100	10
Populus	76	0
Fraxinus	62	14
Carya	58	0
Magnolia	41	0
Total	**1577**	**126**
	31%	**5%**
Total Samples	5002	2744

The difference in the percentages for samples collected for the ten common plant groups shown above is due in part to WBA collecting more herbaceous species. This is further evident in the following table in a comparison of the total number of samples collected from the US by the WBA and by the MA (Morton Arboretum) according to categories of plant parts. The more (frequent) active parts are indicated in bold—**root** (rt, rt-st, rt-rh, wr), **root-bark**, **stem-bark** (sb, inner bark, outer bark, new sb, old sb), **flower and fruit** (with or without inflorescence parts), followed by the less active parts in normal type—woody-stem (including bark, or with bark removed), twig, twig and leaf together, leaf alone, and herbaceous vegetative parts (hst, st, pl, px, rt-st-lf, st-lf). The herbaceous plants represent 26.5% of all WBA samples as determined by samples of the entire plant (pl, rt-st-lf-), or aerial parts without root (px, st-lf-, st).

Plant Parts	**Rt,** rt-st, rt-rh, wr	**rb**	**sb**	**fl-fr**	Ws, wst, ws-sb	tw	tw-lf	lf	hst, st, pl, px, rt-st-lf, st-lf	**Total**
WBA	**668** **24.3%**	**111** **4.0%**	**176** **6.4%**	**131** **4.7%**	350 12.8%	143 5.2%	218 7.9%	219 8.0%	728 26.5%	**2,744**
MA	**925** **18.5%**	**0**	**426** **8.5%**	**141** **2.9%**	1,264 23.5%	1	1,140 22.8%	145 3.2%	1,028 20.5%	**5,002**

Other differences in the above table between the MA and the WBA samples are related to sampling methodology. Under twig (tw) samples, the WBA separated leaf for143 species of woody plants, compared to just one (1) twig sample collected by the MA. The MA leaf samples were observed to be from herbaceous species and also leaf rosette shrubs in the Agavaceae. Another difference is with woody-stem samples (wst, ws-sb, ws); the WBA separated bark from the stem-wood more often than the MA as seen by the lower percentage, 12.8%, compared to 25.2% for MA; however, it is not clear whether the MA submitted a separate sample of woody-stem (ws); their designation "ST" when they had also obtained a "BK" sample from the same species (e.g., madrone, *Arbutus menziesii*), indicates that they (MA) could have collected bark from the trunk, and submitted a branch (with bark attached) as a separate sample, labeling it as "ST."

Recalling the earlier discussion on the NCI collections prior to 1982, the MA and the WBA have more thoroughly sampled plants in the United States than past collectors as evident by the large number of root samples they (MA, WBA) collected. The WBA also obtained samples of root when encountering species or genera that the MA had only collected aerial parts, e.g., *Abronia latifolia, Aloysia, Amsonia, Angelica, Aquilegia, Aralia californica, Arctostaphylos pungens, Buddleja, Celtis pallida, Chlorogalum, Clematis, Condalia, Fraxinus anomala, Mirabilis, Karwinskia, Lepidium, Leptodactylon, Polygala, Prunus fasciculata, Quercus berberidifolia, Rhus microphylla, Rhus ovata, Rhus virens, Salvia ballotaeflora, Ungnadia,* and *Ziziphus*.

Additionally, many species and genera of shrubs in the western US—that the WBA collected—include a separate sample of bark, either from the root, or from the stem, or from both. Genera or species from which bark was collected by the WBA in the western US, but not by previous suppliers, include: *Acacia, Adenostoma, Aesculus californica, Arctostaphylos, Artemisia tridentata, Baccharis, Canotia, Ceanothus, Condalia, Cornus nuttallii, Corylus, Cotoneaster, Cowania, Cytisus, Cercidium, Cercocarpus betuloides, Cneoridium, Condalia, Cytisus, Eriodictyon, Eysenhardtia, Forestiera, Fraxinus anomala, Fremontodendron, Garrya, Genista, Guaiacum, Holacantha, Karwinskia, Larrea, Lupinus, Malacothamnus, Malosma, Mimosa, Myrica, Olneya, Oplopanax, Prunus fasciculata, Prunus emarginata, Psorothamnus, Purshia, Rhamnus crocea, Rhus, Salix exigua, Salix hookeriana,, Salix lasiandra, Sambucus racemosa, Shepherdia, Sophora, Sorbus, Ungnadia, Vaccinium, Vauquelinia*, and *Viburnum.*

The WBA has gone one step further by collecting 111 samples of root-bark, while also obtaining more fl-fr samples—4.7% (131), compared to 2.9% (141) in the MA collection. To the PI's knowledge, root-bark has not been collected by any previous supplier of US samples for the NCI program. The WBA efforts to increase the number of these kinds of samples would hopefully provide the NCI with a greater chance of finding new anticancer drugs. The most frequently active plant parts (rt, wr, rb, sb, fl-fr) make up nearly 40% of all the WBA samples, compared to 30% of the MA samples.

While other collectors refer to "bio-prospecting" in collecting for the NCI or other organization, and compare their chances of success in finding a novel drug to that of wining a lottery, the PI regards the WBA approach as part of a systematic effort to discover novel antitumor agents in plants—a methodical process in eliminating taxa from the NCI search of plant sources for new anticancer drugs, taking into consideration the taxonomy, chemistry, geography, plant parts, fresh vs. dry, ethnobotanical data, previous collections and screening data results of plants. As the PI reported at an Annual Meeting for the Society for Economic Botany in 1995, he does not play the lottery, but he does collect plants for cancer research because he feels he can stack the odds in his favor!

In the WBA reports on collections from Hawaii and Puerto Rico, it was noted that previous collections from these areas were lacking in root and stem-bark samples. Many historical collections for the NCI are nothing more than easy samples—branches from shrubs and trees divided into ws-sb and tw-lf. Spjut in a USDA ARS Memorandum, Nov 6, 1979, for instance, reported that the bulk of woody samples obtained from South Africa were primarily ws-sb and tw-lf. Perdue (1976)[24] recognized the value of collecting root (wr, rb, rt), but in the interest of conservation, he suggested that in a long-term program, twig be collected instead. He indicated that activity may be picked up in twig (tw) and woody-stem (ws-sb) if many species of a genus are screened; however, it should be recognized that a ws-sb sample is collected only when a stem-bark (sb) is not feasible, but not all collectors make an effort to collect bark samples. A further distinction that WBA makes here is that if activity is discovered in a root sample, recollections can always consider fruit as an alternative. Indeed, thalicarpine—that was isolated from root of *Thalictrum dasycarpum*—led to large-scale collection of fruit that turned out to a more economical source of the compound needed for clinical studies. Samples of flower and/or fruit, however, are rarely feasible in quantities required for prescreening as seen by the relatively low percentages in the samples obtained by the MA (2.9%) and the WBA (4.7%).

Of additional significance is that most WBA samples are from plants that grow in semi-arid and Mediterranean climates of the US. For the past ten years, the NCI has focused on screening samples from tropical species as evident from the NCI RFP that shows many tropical genera with numerous extracts screened. Discoveries from the American desert plants appear relatively few due to apparent neglect in screening desert plants as seen by the RFP data.

Shrubs in the American deserts often produce drastically different parts below and above surface; examples are seen in *Amsonia, Bricellia incana, Ephedra, Foresellsia, Lepidospartum, Mortonia, Senna armata, Thamnosma*, etc. In *Thamnosma montana*, the aerial parts are green and loaded with volatile compounds while the underground parts are like an old stump without any detectable odor.[25] Conversely, the roots of some Mimosaceae give off a noxious odor, whereas the aerial parts are non-aromatic, but have thorns or spines that obviously deter grazers. Unlike plants in other climatic zones, the underground and above parts of many perennials and desert shrubs often appear as if they belong to different species. Yet, there is surprisingly little information on their root chemistry.

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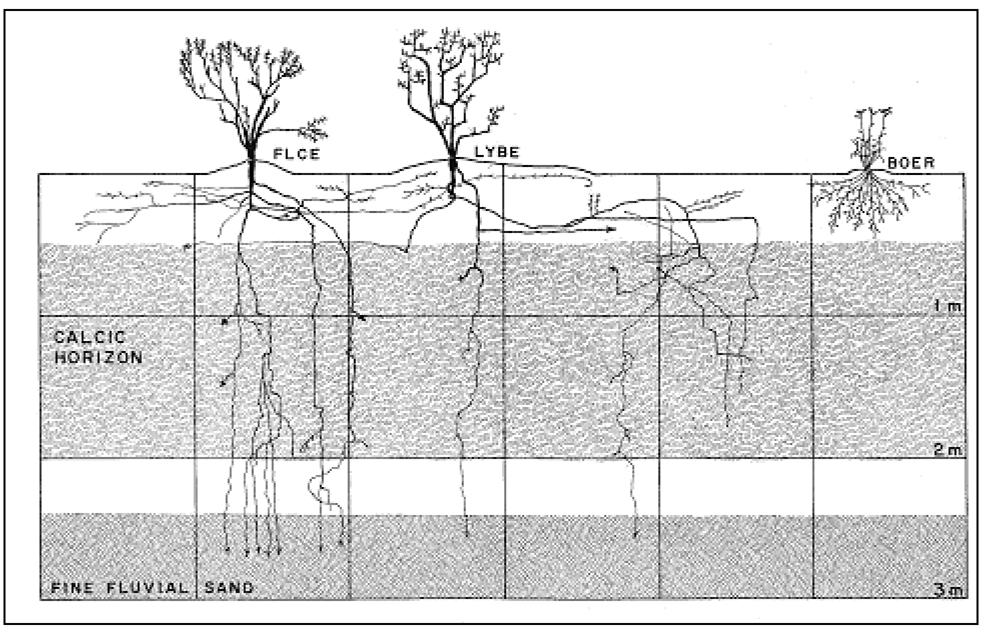
[24] Perdue, R. E., Jr., *Procurement of Plant Materials for Antitumor Screening*, Cancer Treat. Rep. 60: 987–998, 1976.

[25] Furanocoumarins, isopimpinellin and byangelicin, have been isolated from this species, presumably from aerial parts. *The Structures of Phytotoxic Compounds from Thamnosma montana*, D. G. Crosby (1954, Ph.D. thesis). Nonetheless, the roots of another species in Africa, *T. rhodesica*, have been reported to contain 8 furanocoumarins, 1 coumarin and 4 acridone derivatives. Ahua, Kouassi Maximina; Ioset, Jean-Roberta; Ransijn, Adrianab; Mauël, Jacquesb; Mavi, Stevenc; Hostettmann, and Kurt (2004).. *Antileishmanial and antifungal acridone derivatives from the roots of Thamnosma rhodesica*. Phytochemistry 65: 963–968. A novel acridone showed anti-protozoan activity (*Leishmania major*).

The chemical diversity of the desert flora is not only evident from different morphological forms and the variety of detectable odors, but also from personal contact. The PI was able to collect bare-handed in Hawaii samples of plants without any adverse reaction, but when working in the desert he not only had to wear gloves to protect himself from injury by thorns, but also against skin rash. While not particularly sensitive to poison oak or poison ivy, the PI has twice sought emergency treatment as a result of contact from plant species in desert areas, once in Baja California where his arm became grotesquely swollen and another time in the Mojave of Nevada from a strong rash and swelling on his arm. Fortunately, cortisone injections in both cases reduced the swelling.

One might expect that—in competition for water in the desert—plants secrete chemicals from their roots in defense against other species of plants and animals. Allelopathy[26]is certainly recognized in the agricultural fields in the interaction among weeds, crops and insects as evident by the money being provided in grants; for example, plant scientists in conducting biochemical plant studies at Colorado State University have hit pay dirt in receiving 30+ grants in the past 4 years, amounting to ~$5,000,000, with a similar amount reportedly pending, while the Arid Lands Office at the University of Arizona is reportedly screening root rhizophere biota of desert shrubs for novel antitumor and antimicrobial agents.



"Roots of a tarbush shrub (FLCE, *Flourensia cernua*), a Berlandier's wolfberry shrub (LYBE, *Lycium berlandieri*), and black grama (BOER, *Bouteloua eriopoda*) grass growing in a fine-loamy Typic Calciorthid, Dona Ana. Roots ending in arrows were not followed further. The layer of fine fluvial sand prevented following roots deeper. Horizontal distance between tarbush and wolfberry plants is as occurred in field. Plant tops are drawn at same scale as roots." Reproduced from website, with reference to Gibbens RP and J. M. Lenz JM. 2001. *Root systems of some Chihuahuan Desert plants*. J. Arid Environ. 49(2): 221-263.

Collecting root samples can be a sensitive issue. Some permits limit digging to no more than six inches. With huge sums of money being awarded to study chemical interactions between plant roots and their surrounding environment, one would hope that such policies allow collecting to extend 50 cm below surface in order to more effectively collect root samples for research in the NCI drug discovery program. Two scientists in the ARS show that species such as *Larrea tridentata*, *Koberlinia spinosa, Flourensia cernua, Sphaeralcea hastata, Croton pottsii, Lycium berlandieri* have root profiles that penetrate to 5 m or more below surface; one of their many illustrations is shown above

Also, it is not easy to collect root from many desert shrubs because of wide-spreading thorny or sharply pointed branches that make it difficult to get near the base of the plant. Richard Marin of WBA has become quite proficient at getting to the root of the plant, and in removing bark from root as small as 5 mm in diameter. This can often be accomplished without harming the plant. Many WBA root samples were further divided aerial root, root <2mm diam. (also referred to as rootlet), burl, rhizome (underground stem), and adventitious root, depending on the species; examples can be found for *Cneoridium dumosum* (Rutaceae), *Lepidospartum squamatum* (Asteraceae), *Psidium cattleianum* (Myrtaceae), *Rhodomyrtus tomentosa* (Myrtaceae), etc.

Besides the woody flora, the herbaceous plants of semi-arid regions are also more likely to yield bioactive agents than those of wet humid forests as seen in the table below that shows a correlation in antitumor activity for plants collected from wet to dry vegetation types. These data were complied during 1978–1979 by Spjut who has field experience in the areas of study where in Africa he recollected plants obtained originally by Perdue, and in the Sonoran Desert he collected the original (general) samples. As indicated in previous reports, data were reported in memoranda and at scientific meetings, and also received approval from the ARS for publication.

Herbaceous plants aerial parts (pl, px)	% Active in P-388 and/or KB
Kenya Montane Rain Forest	**1.3**
Tanzania Semi-dry Montane Forest	**3.1**
Kenya Lowland Semi-dry Forest	**4.5**
Tanzania Grassland/Woodland	**6.1**
Sonoran Desert	**7.4**

Any one statistical comparison is questionable because of the low frequency of antitumor activity in the NCI screen, but collectively, the data suggest that the chances of finding new leads are greatest with plants that have a long history of adaptation to semi-arid and Mediterranean regions. The American southwest is especially rich in annuals and semi-woody plants; the Cape Fynbos is known for its geophytes and other herbs, and Southwestern Australia for its heath-lands. The Mediterranean plants of California, Chile,

[26] Allelopathy is derived from the Greek words “allelo” and “pathy” meaning reciprocal sufferings of two organisms, which in modern usage refers to direct or indirect (harmful) effect of one plant on another by release of chemical compounds.

Europe might be expected to yield novel compounds that may differ from secondary metabolites of their ancestral relatives in mesic forests. In the drier desert areas of northern Africa, southwest Asia and central Australia, one might expect to see fewer novel compounds because of the more recent origin of the plant life. The 58 floristic regions recognized by Spjut (1985, l.c.) takes these differences into consideration. Notwithstanding, tropical forests are richer in species, but discovery of their novel active compounds requires screening more samples as related to a lower incidence of activity.

Spjut (1985, l.c.) also suggested a point of diminishing returns not only for the number of species in genera but also for sampling the floral kingdoms of the world. In regard to the limitations to collecting species as encountered in selected geographical areas, after a cumulative number of species have been screened, it may be worthwhile to go after selected genera and species in order to discover new compounds—as Spjut did for the earlier NCI program (Spjut 1985, l.c.). Further consideration may be given to whether plant orders and families such as the Cucurbitales, Simourbaceae, and Thymelaeaceae will yield active compounds other than what they already are known to produce, cucurbitacins, quassinoids, and daphane diterpenoids, respectively.

Identification and Distribution of Voucher Specimens

The WBA has been fortunate in receiving collaborative assistance from the Botanical Research Institute of Texas (BRIT) for plant identifications. Roger Sanders, in reviewing the identifications, frequently provided useful taxonomic remarks in reference to our collection numbers and identifications. We also appreciate the identifications by Guy Nesom for the many species of Asteraceae from the southwest and from other areas. Vouchers have been sent to BRIT, the US National Herbarium (US), and the University of California Herbarium at Berkeley. The WBA retains one set, which have been mounted and scanned by Sally Larsen at Fallbrook, California, and filed in the WBA herbarium in Bakersfield. Additional vouchers are also at our Pala facility, and National Park facilities at Big Bend and at Amistad National Recreation Area.

Specimens were scanned to help the PI avoid duplication in the 4th and 5th years of the contract. The WBA is placing the scanned images online at www.worldbotanical.com, under Photos of North American plants, which may include photos taken in the field. Other historical data are planned for these pages such as botanical data from more 200 reports on active species, and as many as 3,000 slides on plants collected in Africa, Australia (Tasmania, Western Australia), Mexico, Galapagos Islands, Hawaii and Puerto Rico. These include special collections involving the Boy Scouts of America for *Colubrina california*, large-scale recollections of *Baccharis megapotatmica, Maytenus buchananii, Bouvardia ternifolia, Thalictrum dasycarpum*, cultivation of *Camptotheca* at Chico, California, and also procurement of *Taxus brevifolia* sb. Currently, the WBA web page presents keys and descriptions to all species of *Taxus*, accompanied by photos of herbarium specimens and types, a key to lichen species of *Niebla* for Baja California with images of specimens for each species, an outline of the different kinds of fruits with links to images of fruit types and discussion of the problem with fruit terminology, and the history of the WBA.

Abbreviations for Plant Parts and Numbers Assigned to Plant Samples

Plant samples are usually labeled in the field with collector's number, scientific name, and plant parts, which are abbreviated according to standards developed by the ARS and the NCI as published in Cancer Chemotherapy Screening Data summaries during the 1960's, and by Perdue (1976).[27] We have modified the plant part designations slightly to distinguish between herbaceous stems (hst) and woody stems (wst) of semi-woody plants, and occasionally capitalize designations when the sample consists primarily of one part with other parts included (for the WBA database).

In addition to the collector's (voucher) number, samples are also assigned a WBA accession number for each collection, and a NCI number for each sample using the bar code labels provided by the NCI. Many chemists who have in the past published on the chemistry of a plant sample, have often cited the accession number (PR numbers, WBA numbers) when they should have cited the collector's (voucher) number. Accession numbers, which are assigned after all samples have been dried and assembled for shipment, do not identify a voucher specimen, only a sample of a number of samples that may have been collected from one species at the time of gathering. The NCI previously recorded the PR accession number in their database prior to 1986, but the corresponding WBA accession numbers were not entered in the present contract because there is no data field for this number; we only provided the collector's (voucher) number for a specimen that is linked to the NCI (Q73H) automated number for each sample.

Since May 2002, the WBA has been entering all collection data into the NCI database, online. Data were transferred by copying each field of data from the WBA database into the corresponding field of the NCI database, except for pull-down menus that require selection from a list of NCI terms such as the designations for plant parts. Since our method of labeling plant parts differs from that of the current NCI format, in which we have followed the former NCI format, we have selected the designation that best matched our sample. Any need for more detail on plant parts collected can be found on the shipping list that was sent to the Project Officer, and in the "comments" field of the NCI database.

Our method of accessioning follows that which was used by the USDA ARS for 22 years (1960–1982), except for annexing a two-digit number to each accession as explained below. For each shipment we provided a print out of a shipping list that summarized the collection data, and included the weight of the sample to the nearest 10 g.[28] Samples collected in the mainland were shipped from our facility in Pala,

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[27] Perdue, R. E., Jr., *Procurement of Plant Materials for Antitumor Screening*, Cancer Treat. Rep. 60: 987–998, 1976. The NCI recently has modified the abbreviations for their data entry purposes based on codes also developed by other suppliers..

[28] The ARS assigned a "PR" number to each plant sample sequentially from 1 to more than 58,000 that were accessioned up to October 31, 1982, whether a general sample or a recollection, and also to another numerical series starting at 80,000 for plants sampled in large quantities that were suspected to contain active compounds (e,g, Apocynaceae, Celastraceae, Rutaceae, Colubrina). Data associated with the PR

California. Those from Alaska, Hawaii, and Puerto Rico were shipped from those locations. At remote locations, collection data were entered into our lap top computer from which shipping list, voucher labels, and other reports were prepared. These data were then transferred to our desk top computer and then to the NCI database.

Because of reidentifications by BRIT that were made after shipment of samples to the NCI repository, occasionally, the name on the bag may not agree with that in the NCI database in which case the collection number may need to be consulted if need for further inquiry.

Also, the WBA accession number written on the outside of each sample bag (attached label tag) indicates how many samples there are from a single collection (of a species). For instance, a root sample of *Montanoa hibiscifolia* (Asteraceae) from Hawaii that was accessioned as WBA 5115-15 indicates 5 samples were collected by the last digit of the number (5) following the dash, and that this is the first of the five samples as indicated by preceding digit (1) of that number; a leaf sample that was the fifth and final sample of that series was accessioned as WBA 5115-55. Samples are routinely accessioned starting from the lower plant parts, progressing towards the upper parts. The next species we accessioned was *Ipomoea alba* (Convolvulaceae) for which we only collected one sample, st-lf-fl; it was accessioned as WBA-5116-11.

Fresh Samples

Last October, the Project Officer suggested to the PI that a chemical evaluation of fresh vs. dried samples be undertaken based on a sampling of 30 species. The PI felt more species may be required, but due to termination notice in March, we were only able to provide samples from 24 species. Samples were collected in quantities to yield 250 g dried; a scale was used to weigh most samples at the time of collection. These were collected from *Abronia villosa* pl (Nyctaginaceae), *Allenrolfea occidentalis* green tw

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numbers were maintained as a final typed shipping list in three-ring binders, generally ten records (accessions) to a page, 1,000 accessions (100 pages) to a binder. Each PR number was followed by information on plant parts, sample weight, state or country of collection, abbreviation for the collector with his or her number, date of collection, and date of shipment. This was prepared on a magnetic tape (type-writer) with the intent for transfer to a computer file—at the National Agricultural Library (NAL). However, when Spjut inquired at NAL about this during the 1990's, it seems that no-one at NAL could recall anything about the tape or accession records, although he recalls discussions from NAL personnel on the matter during the 1970's. The only link between the voucher specimen and the plant sample is to be found in the ARS accession records, except for active plants; it was not until the late 1970's when NCI added the collector's number to their file. The collector's name was abbreviated for ARS employees and routine suppliers, and the occasional collector was designated M (for Miscellaneous); however, identifying the miscellaneous collectors can only be determined by the assigned collection number that was kept in a another file that consisted of small, standard, green field notebooks, which probably were discarded in March 1983 with the thousands of voucher samples (active plants) that were also discarded at that time. The whereabouts of the original printout of the accession records is unknown. In Goodman and Walsh, *The Story of Taxol* (2001), reference to "PER" for the date of shipment of *Taxus brevifolia* sb implies that Perdue has a copy of the accession records—at least for that of *Taxus brevifolia*. One other file known as "active books"—that recorded the ARS collection data for each NCI active species in 3-ring binders, organized by state or country—were retrieved by Spjut from Frederick MD and deposited into the NAL with a memo to Al Fusonie.

(Chenopodiaceae), *Ambrosia dumosa* if (Asteraceae), *Atriplex hymenelytra* tw-lf (Chenopodiaceae), *Chaenactis steveoides* pl (Asteraceae), *Coreopsis bigelovii* pl (Asteraceae), *Coronopus didymus* pl (Brassicaceae), *Cryptantha angustifolia* (Boraginaceae), *Cuscuta indecora* px (Cuscutaceae), *Eruca vesicaria* pl (Brassicaceae), *Ferocactus cylindraceus* st (Cactaceae), *Isomeris arborea* tw-lf (Capparaceae), *Larrea tridentata* tw-lf-fl bud (Zygophyllacae), *Lepidium fremontii* tw-lf-fl (Brassicaceae), *Malacothrix glabrata* pl (Asteraceae), *Oenothera deltoides* pl (Onagraceae), *Peucephyllum schottii* tw-lf (Asteraceae), *Phacelia tanacetifolia* (Hydrophyllaceae), *Platystemon californicus* pl (Papaveraceae), *Portulaca oleacea* pl (Portulaceae), *Rafinesquia neomexicana* pl (Asteraceae), *Salvia columbariae* pl (Lamiaceae), *Streptanthella longirostris* pl (Brassicaceae), and *Yucca brevifolia* fl (Agavaceae).

It was observed that some samples, particularly Asteraceae, mature as they dry. An inflorescence sample of *Ambrosia dumosa*, for instance, developed the nasty prickles when dried; thus, while the fresh sample was flower, the dried sample was mostly fruit when shipped.

Fresh samples were placed on dry ice, or occasionally in a freezer if collected not far from the office. Fresh samples were accessioned and then shipped in coolers on dry ice via overnight express to Frederick, MD. Dry ice was obtained in Bakersfield, Las Vegas, NV, and Yuma, AZ. Accession numbers were assigned consecutively to both samples when the first of two was to be shipped fresh. In discussion with Dave Newman at NCI (the Project Officer was away on a trip), it was decided that the fresh sample be given an odd number, the dried an even number. Shipping list includes the fresh weight for dried samples in parenthesis.

Future Collections from the US

The NCI terminated the contract for the collection of US samples before the WBA completed its proposed work. The NCI budget reductions reportedly led them to focus more on drug development of the leads they now have. There are still many new species and genera to be collected in the Pacific Northwest, Inter-Mountain and Rocky Mountains regions. All the MA collection sites in western states were plotted on state maps indicating the number of species collected and month of collection.

There are some fairly common semi-woody species such as *Encelia californica* (Asteraceae) that were not sampled by either the Morton Arboretum or the WBA. The PI has seen *E. californica* many times but has not bothered to collect it because he was waiting for other species to develop in the same proximity. Although, the WBA was able to collect samples from many annual species, there are still many that were not collected because the western region has generally been under a drought during our contract period.

In future biodiversity type collections, one would hope that permits could be issued from a national level rather than having to apply to each forest district and each national park. The WBA recognizes that the local botanists for each of the many regions are trained to look for and protect their rare species, but it appeared to the PI that some botanists are sensitive to any plant being collected for any purpose but their own. In one area of on the mainland, nearly 50% of the species were classified as rare. The justification for preserving our natural plants includes the possibility that some plants may contain novel chemicals for treating human diseases. The fact that this work is being done by our government and is of value to humankind—for cancer—would lead one to expect cooperation in obtaining permits, but from the WBA experience, it seems that some care more about the monetary returns and agreements as firmly held by one scientist in charge of a natural area in California. The management practices that vary considerably from region to region, along with permit conditions, make it difficult for reporting as well as trying to keep track as to what is allowed and what is not allowed.

The PI wishes he could have interacted more with local botanists, but budgetary constraints and time limitations required him to spend his time in the field; he did not allow much for administrative activities as he did in the late 1970's when field work involved both selective and biodiversity sampling strategies. In any case, the WBA expresses its appreciation to the grantors of permits, especially to the Big Bend National Park in advising where to find plant species, the use of facilities provided by the Chugach National Forest in Alaska and their suggestions on where to collect in the forest, the Chief Forester's assistance in the Maricao State Forest Reserve in Puerto Rico along with his assistant and use of his vehicles and tools, the maps provided by the Klamath National Forest and the Eldorado National Forest, administrative passes from the Angeles National Forest, the San Bernardino National Forest, and the Cleveland National Forest, the longer term permits provided by the BLM California, the Coronado National Forest, and the Angeles National Forest, and the wider geographical coverage provided by permits from the regional national forests in the Pacific Northwest, Rocky Mountains, and the Caribbean National Forest for allowing access inside gated road, and finally, the expedient processing of permit applications by the Sierra National Forest, Six Rivers National Forest, Modoc National Forest, and Inyo National Forest.

The WBA looks forward to any future opportunities for collection of samples for the NCI.