Limitations of a Random Screen: Search for New Anticancer Drugs in Higher Plants[1]

RICHARD W. SPJUT[2]

The inherent limitations of a random search of higher plants for novel cancer chemotherapeutic agents are reviewed—the National Cancer Institute's (NCI) Anticancer Screening Program. A graphic summary of plant exploration for the NCI is depicted on a world map showing 58 floristic regions. It is estimated that less than one-half of the world flora is economically feasible for collection. Random screening of approximately 35,000 species has led to guidelines that precluded further screening of all species in 333 genera and another 2,905 species in 1,773 genera. These taxa are reported to represent one-half to two-thirds of the species that characterize vegetation in geographic areas most frequently explored for the NCI. It is estimated that 40,000 untested species of flowering plants are readily available and meet the NCI guidelines for antitumor screening. However, because of apparent diminishing returns from random screening of chemicals in plant genera, it is suggested that a good representation of the diversity in the world flora could be obtained in 10,000 collections, if random sampling follows the phytogeographic outline that is recommended. Modifications to the screening methodology might be geared to an expected point of diminishing returns for discovering novel chemotypes. Additionally, the NCI should continue random screening to increase the development of new anticancer drugs; past screening has generated a tremendous wealth of data. Finally, in this paper, the author proposes to utilize lists representing taxa commonly collected for the NCI to create a manual of worldwide common plants.

In 25 yr, the National Cancer Institute (NCI) screened more than 120,000 plant extracts from 35,000 species for novel anticancer agents. Some promising discoveries are: taxol, indicine-n-oxide, phyllanthoside, and homoharringtonine, isolated from *Taxus brevifolia* Nutt., *Heliotropium indicum* L., *Phyllanthus acuminatus* Vahl, and *Cephalotaxus harringtonia* (Knight ex Forbes) K. Koch, respectively (M. Suffness, pers. comm.).

From 1960 until 1982, about ½ of the plant samples were supplied to the NCI through a cooperative agreement with the Agricultural Research Service (ARS) of the United States Department of Agriculture (USDA). This agreement, expending nearly ½ million dollars annually since 1972, was terminated as a result of widespread 1981 budget cuts of federal programs. Other substantial suppliers were Commonweath Scientific and Industrial Research Organization (Australia), Central Drug Research Institute (India), National Defense Medical Center (Taiwan), University of Arizona, University of Costa Rica, University of Concepcion (Chile), University of Brazil (Rio de Janeiro), and the University of Hawaii (J. L. Hartwell, pers. comm.).

---

[1] Received 3 February 1984; accepted 19 January 1985.

[2] Manuscript prepared privately, a contribution of World Botanical Associates, P.O. Box 2829, Laurel, MD 20708-0829. The views expressed and interpretation given are those of the author and do not reflect the official view of the ARS, USDA.

The NCI procedure followed a stepwise-collecting and -testing protocol to isolate methodically bioactive chemicals in plants for ultimate evaluation in the treatment of human cancers. Initially, species were randomly collected in small amounts from ½–2 kg dried weight. Samples of roots, bark, twigs, leaves, or any combination of these were submitted to a routine extraction and prescreening procedure (Suffness and Douros, 1979). Extracts from approximately 10% of 35,000 species tested were active, i.e., these extracts significantly inhibited tumor growth, increased the life span of leukemic mice, and/or were cytotoxic in vitro (Geran et al., 1972). Selected active species were then re-collected in large quantities (50–250 kg dried) to isolate the active agent(s). Occasionally, massive samples of one to many tons were needed to supply sufficient amounts of the active agent for preclinical and/or clinical studies.

In this paper, the terms "tested" and "screened" refer to the "prescreen" procedures (Suffness and Douros, 1982). Abbreviations cited for tumors (screens, bioassays, or test systems) follow Hartwell (1976, Table 2), and include: WA (Walker carcinoma 256, rat), SA (Sarcoma 180, mouse), CA (Adenocarcinoma 755, mouse), KB (Human epidermoid carcinoma of the nasopharynx, cell culture), LE (Lymphoid leukemia L-1210, mouse), LL (Lewis lung carcinoma, mouse), and PS (Lymphocytic leukemia P-388, mouse). The duration for which these were employed in the prescreen is shown in Suffness and Douros (1979, Fig. 2); PS and KB were the tumors primarily used since 1969.

"Random" collecting was not entirely random (Spjut and Perdue, 1976). It is broadly defined in this paper as sampling without a preconceived selection of species. This is not to imply that samples were obtained without thought. An initial tendency was to shortcut the discovery process by collecting plants on the basis of folkloric, chemotaxonomic, and climatic relationships, but, as screening experience progressed, there was a tendency to minimize bias in collecting so that promising compounds would not be missed. Guidelines focused more on excluding rather than selecting plant taxa—species in 1971, families to a limited extent from 1972–1975, and, finally, genera in 1979. Taxa were precluded from further screening for 2 reasons: (1) many samples had been tested without yielding significant activity, or (2) active agents had been isolated and it was apparent that continued screening would not lead to isolation of new compounds. Unless a change was made in the screening methodology, there was little to gain from collecting additional species of figs (*Ficus*), for example, because after testing more than 10% of the species in *Ficus*, it was clearly evident that PS activity was infrequent and unlikely to exceed marginal criteria; similarly in milkweeds (*Asclepias*) activity would predictably occur in KB, and the cytotoxic agents were invariably found to be cardenolides. Genera excluded from further screening are listed in this paper (Table 1, 2).

The random acquisition of higher plant samples was thus carefully guided to avoid duplication of those genera and species already screened. With the exception of excluded plants and those not occurring in sufficient abundance, species were sampled as encountered in selected geographic areas. Additionally, plants in genera not previously tested and others reportedly used for certain medicinal purposes were especially sought out but "random" collecting was not entirely abandoned in lieu of this. These modalities were sometimes combined into an overall strategy

TABLE 1. GENERA WITH 100 OR MORE EXTRACTS SCREENED FOR ANTITUMOR ACTIVITY.[a]

Abies—50
Abutilon—100
Acacia—800
Acalypha—450
Acer—200
Aegiphila—160
Agave—300
Albizia—150
Alchornea—70
Allium—450
Allophylus—190
Alnus—35
Aloe—332
Amaranthus—60
Annona—120
Ardisia—400
Artemisia—400
Asclepias—120
Aspidosperma—80
Aster—500
Astragalus—2,000
Atriplex—200
Baccharis—400
Bauhinia—300
Berberis—450
Betula—60
Bidens—230
Bridelia—60
Buddleja—100
Byrsonima—120
Caesalpinia—100
Calliandra—100
Calyptranthes—100
Canthium—200
Capparis—250
Casearia—160
Cassia—600
Cassine—40
Casuarina—45
Ceanothus—55
Cecropia—100
Celtis—80
Centaurea—600
Cestrum—150
Chenopodium—150
Chrysanthemum—200
Chrysophyllum—150
Cinnamomum—250
Cirsium—150
Cissus—350
Citharexylum—115
Citrus—12
Clematis—250
Clerodendrum—400
Clethra—68
Clusia—145
Coccoloba—150
Combretum—250
Cordia—250
Cornus—4
Crotalaria—650
Croton—750
Cryptocarya—250
Cupania—55
Cyperus—550
Dalbergia—300
Datura—10
Derris—80
Desmodium—450
Dioscorea—600
Diospyros—500
Dombeya—350
Drypetes—200
Elaeocarpus—200
Erigeron—200
Erythrina—100
Erythroxylum—250
Eucalyptus—500
Euclea—20
Eugenia—1,000
Euonymus—176
Eupatorium—1,200
Euphorbia—2,000
Fagara—250
Faramea—120
Ficus—800
Fraxinus—70
Garcinia—400
Gardenia—250
Gnidia—100
Grewia—150
Guarea—170
Gaultheria—250
Helenium—40
Helianthus—110
Helichrysum—500
Heliotropium—250
Hibiscus—300
Hypericum—400
Hyptis—400
Ilex—400
Indigofera—700
Inga—200
Ipomoea—500
Jacaranda—50
Jasminum—300
Jatropha—175
Juniperus—60
Lantana—150
Liatris—40
Linum—230
Litsea—400
Lobelia—300
Lonchocarpus—150
Lonicera—200
Lupinus—200
Macaranga—280
Manilkara—70
Maytenus—225
Miconia—700
Mikania—252
Mimosa—500
Morinda—80
Myrcia—500
Myrica—35
Nectandra—100
Ocotea—400
Oenothera—80
Opuntia—250
Palicourea—200
Passiflora—500
Penstemon—252
Persea—150
Phoradendron—190
Phyllanthus—600
Physalis—100
Phytolacca—35
Pinus—100
Piper—2,000
Pithecellobium—200
Pittosporum—150
Plantago—265
Pluchea—50
Podocarpus—100
Polygonum—300
Populus—35
Potentilla—500
Pouteria—50
Protea—130
Protium—90
Prunus—430
Psidium—140
Psychotria—700
Quercus—450
Randia—300
Rapanea—200
Rhamnus—160
Rhus—250
Rosa—250
Rubus—250
Rumex—200
Salix—500
Salvia—700
Sambucus—40
Sapium—120
Scaevola—100
Senecio—3,000
Sida—200
Siparuna—150
Sloanea—120
Smilax—350
Solanum—1,700
Solidago—100
Sterculia—300
Strychnos—200
Styrax—130
Swartzia—100
Symplocos—350
Syzygium—500
Tabebuia—100
Tabernaemontana—100
Tecoma—16
Tephrosia—300
Terminalia—250
Thalictrum—150
Theobroma—30
Tibouchina—200
Tournefortia—150
Trema—30
Trichilia—300
Vaccinium—400
Verbena—253
Vernonia—1,000
Viburnum—216
Virola—60
Vismia—35
Vitex—250
Vitis—70
Xylopia—150
Yucca—40
Zanthoxylum—30

Total: 201 genera
58,956 species

[a] Numbers following genera are of species in genus (Willis, 1973).

of random collecting that was targeted for geographical areas predetermined to have high concentrations of untested genera and preselected medicinal plants (USDA Memorandum, 1979c).

Retrospective studies on the relationships of antitumor activity with taxonomy