UNITED STATES DEPARTMENT OF AGRICULTURE
AGRICULTURAL RESEARCH SERVICE

APPLICATION FOR APPROVAL TO ENGAGE IN OUTSIDE EMPLOYMENT OR ACTIVITY

Vascular Plants + travel CV.

SECTION I – GENERAL INFORMATION *(To be completed by the employee)*

A. Agency Employment

NAME	LOCATION
RICHARD SPJUT	BARC-East, Bldg. 265 Beltsville, MD 20705
POSITION TITLE & GRADE: Support Scientist, Botanist, GS-9	
DAYS OF NORMAL WORKWEEK	OFFICIAL DUTY HOURS

B. Prospective Outside Employment or Activity

NAME & ADDRESS OF PROSPECTIVE EMPLOYER	DESCRIPTION OF WORK, DATES OF EMPLOYMENT, & LOCATION
World Botanical Associates P.O. Box 2829 Laurel, MD 20708-0829	Collect up to 300 samples of wild edible plants from the US for chemopreventative screening.

PAY, REIMBURSEMENT, OR TERMS OFFERED				
$40/sample			Will this activity involve use of unpublished research or information not publically available?	Yes: ; No: X
Will Annual Leave or Leave without Pay be necessary?	Yes: X	No:	Do you have any contact with the prospective employer or activity in your official capacity?	Yes: ; No: X
Will this activity interfere with your official duties?	Yes:	No: X		

C. Additional Comments

(Use this space to provide any additional explanations, details, or other information you feel is pertinent. Additional sheets may be attached.)

Samples will weigh from 400-500 g and will be screened by Dr. John Cassady at Ohio State University.

D. Certification

I certify that the information provided above is complete and correct to the best of my knowledge. I further certify that I have reviewed and am familiar with the provisions of Appendix I, of the Employee Responsibilities and Conduct Handbook, with respect to outside employment and activities.

SIGNATURE: Richard Spjut

DATE: April 11, 1988

SECTION II – REVIEW AND APPROVAL *(To be completed by reviewing officials.)*

A. Comments

The immediate supervisor should indicate reasons for recommending disapproval – – or reasons for recommending approval when deemed necessary. Additional sheets may be attached.

B. Final Action

	SIGNATURE & TITLE *(Immediate Supervisor)*	DATE
☒ APPROVAL RECOMMENDED ☐ NOT RECOMMENDED	Amy Y. Rossman, RL, SBMNL	4-15-88
☒ APPROVED ☐ DENIED	K Mansell	5/15/88

Employee Copy

History of Plant Collecting in U.S.

of the Cape Mountains along roads not shown on any maps, and several islands, Isla Cedros and Isla Santa Margarita.

1988 (Mar). WBA expedition--- 200 lichen specimens with photographs, primarily *Niebla spp.*; 17 general samples of vascular plants for chemopreventive screening (OHSU). Exploration was mainly along the Pacific Coast between San Quintin and Punta Santa Rosalillita.

1986 (May). WBA-Purdue University Expedition--103 general samples (1 kg/sample) and ~ 10 re-collections of vascular plants (7 kg/sample) for antitumor screening. Also, ~ 1,200 collections towards lichen flora of Baja California, ~ 30% in *Niebla* and one other related genus (unpublished). Exploration covered the entire peninsula of Baja California.

1985 (May). WBA travel--- 50 samples of lichens for the NCI antitiumor and anti-AIDS screening (25-100g/sample); received assistance in identifications from Mason Hale for Parmeliaceae, Clifford Wetmore for Collemataceae, Richard Harris for *Lepraria* and *Chrysothrix* and William Weber for Roccellaceae. Exploration covered the Pacific Coast from Tijuana to San Antonio Del Mar and Punta Negra to Punta Santa Rosalillita, and the San Pedro Martir.

1980 (Mar-May). USDA expedition-- ~ 200 general samples (1.5-2kg/sample) for antitumor screening (NCI). Exploration covered the entire peninsula; most collections were obtained from the southern half of the peninsula.

1979 (Feb-Apr). USDA expedition-- ~ 150 general samples (1.5-2kg/sample) for antitumor screening (NCI); exploration covered the entire peninsula.

Coahuilla. 1976 (Aug). USDA collection--5 tons (dried) of *Bouvardia ternifolia*, in collaboration with an ARS supplier.
Jalisco. 1980 (May). USDA, part of expedition to Baja California--- 50 general samples (1-2 kg) for antitumor screening, primarily between Puerto Vallarta and Guadalajara on back roads; received assistance in identification from Sandra Saufferer.

VIII-E EUROPE

England. 1973 (Mar), **1976** (Oct). USDA travel--Kew Gardens Herbarium and British Museum of Natural History for geographic data on plants with known antitumor activity.

VIII-F UNITED STATES AND CANADA

1995. May--SEUS: 88 general samples for PU primarily from Florida, Texas and New Mexico, but also scattered collections from Mississippi, Arkansas, Oklahoma, and Tennessee; 5 recollections, 5-10 kg each from Florida, Arkansas and West Virginia for OHSU; 1 special collection for preclinical studies of anticancer compounds.

1994. Oct--AZ: Recollection of species for chemical undergoing preclinical evaluation against slow-growing tumors. **Sep--Great Lakes region**: approx. 20 general samples of vascular plants, 100-500 g for antitumor screening at PU; recollection of 1 bryophytes and 1 vascular plant species for VPSI. **Jul--Pac NW**: Recollections of 1 fern and 2 bryophytes, and 50 general samples from California, Oregon, Washington, Idaho, Montana, and Nevada for PU, OHSU, and VPISU.

1993. Oct--Michigan: recollection of a moss. **Jul--California:** WBA re-collections of mosses and ferns for antitumor research; about 100 general samples obtained for screening by VPISU, OHSU, and PU. **May--North Carolina:** 5 kg recollections of two species of moss.

1992. Jul-Aug--Pac. NW: WBA re-collections of lichens and mosses for antitumor and anti-AIDS research; taxonomic study of *Taxus*, one new species of *Taxus* is being recognized by its habit and vegetative manner of reproduction.

1991. Jul-Aug--NUS & Canada: WBA--67 general samples (100+ g/sample) of vascular plants (PU & OHSU) and 38 of bryophytes (VPISU) for antitumor screening, 1-5 kg samples of yews and the Californian nutmeg (*Taxus brevifolia* both shrub and tree variants, *T. canadensis, T. cuspidata, Torreya californica*) for analysis of taxane compounds (VPISU), 1-2 kg lichen re- collections of *Cladina arbuscula, Peltigera canina* and *Xanthoparmelia cumberlandia* for antitumor screening (NCI), 1-5 kg bryophyte recollections of *Dicranum ontariense, Lescuraea incurvata, Pellia epiphylla, Ptilidium ciliare* (VPISU), 5 kg vascular plant recollections of *Betula papyrifera* (PU). *Comptonia peregrina*(PU), *Eriodictyon californicum* (OHSU), *Eriogonum microthecum* (UMS). **May--Nevada:**

22 general samples (100+g) of vascular plants for antitumor screening (OHSU & PU), 5 kg re-collection of *Stanleya pinnata* for antibiotic screening(UMS). **Jun--Maryland (Cumberland):** 7 bryophytes samples (100+g, VPISU) for antitumor screening. Received some assistance from Neil Harriman; vouchers of vascular plants were deposited at Oshkosh (OSH)

1990. Dec--Nevada & Arizona: WBA 5 kg re-collections of *Peucephyllum schottii, Forsellesia nevadensis, Fallugia paradoxa* for antibiotic screening (UMS). **Oct--Tennessee (The Great Smoky Mountains National Park), New York (Letchworth State Park) and Pennsylvania (Alleghany National Forest):** 30 selective bryophyte samples (100 g/sample) for antitumor screening (VPISU), participated in the American Bryological and Lichenological Society Foray. **Sep--California:** special collections of *Taxus brevifolia* seed, bark (5 kg) and root (5 kg), and live plants; re-collections (5 kg) of vascular plants (3 spp., PU); 10 general samples (100 g/sample) of bryophytes and vascular plants for antitumor screening (VPISU, PU). **Aug--New Hampshire (White Mountains National Forest):** 33 kg re-collection of a moss (*Polytrichum pallidisetum*) for antitumor screening (OHSU); 60 general samples (100 g/sample) of bryophytes for antitumor screening (VPISU); live and dried samples of *Taxus canadensis* for tissue culture and antitumor screening (PU). **Jun-Jul--Pacific Northwest:** 100 bryophyte samples (100 g/sample) for antitumor screening (VPISU); 5 re-collections of vascular plants (5 kg/sample) for antitumor screening (PU). **Apr-Southwestern U.S. (Texas, Nevada, California, Arizona):** 5 re-collections (5 kg/sample) and 30 general samples (100 g/sample) of vascular plants for antitumor screening and chemopreventive screening (PU, OHSU). **Mar & Jul--Washington:** WBA inspector of western yew bark (*Taxus brevifolia*) for the NCI; collected bryophytes samples near Seattle for antitumor screening (VPISU).

1989. Nov 8-11--Washington: WBA inspector of western yew bark (*Taxus brevifolia*) for the NCI and 20 samples (100 g/sample) of bryophytes for antitumor screening (VPISU). **Aug 25- Oct 16, WBA travel by auto across the U.S.:** 150+ samples (100-500 g/sample) of vascular plants for chemopreventive and antitumor screening (PU, OHSU), and 20 re-collections (1 kg/sample) of lichens for anti-AIDS screening (NCI). **Jul--North Carolina and Virginia:** 3 lichen WBA re- collections (1-5 kg/sample) for anti-AIDS screening (NCI).

1988. Sep-Vermont: participated in the American Bryological and Lichenological Society Foray. **Jul--California and Oregon:** WBA procurement-- ~ 70 samples of edible and medicinal plants for chemopreventive and antitumor screening (PU); WBA consultant and inspector of bark of 30 tons of western yew, (*Taxus brevifolia*, NCI).

1987. Sep-Oct--Maryland, Virginia and New Jersey: WBA 20 general samples of edible plants (500 g/sample) (PU), **May--California and Nevada:** WBA re-collections of vascular plants (*Cercocarpus betuloides* (8 kg rt) and *Cryptantha confertiflora* (10 kg whole plant). Maryland (PU) 20 general samples of edible plants (PU).

1986. Jul--Eastern U.S. (North Carolina to Maine and Wisconsin): WBA re-collections of bryophytes and lichens for antitumor screening at VPISU (*Anomodon attenuatus* 50 kg, *Bazzania trilobata* 5 kg, *Brachythecium oxycladon* 1 kg, *Dicranum fulvum* 22 kg, *Hylocomium splendens* 23 kg, *Peltigera canina* 3 kg, *Peltigera elizabethae* 1 kg, *Polytrichum pallidisetum* 6 kg, *Plagiomnium ciliare* 1 kg, *Ptilium crista-castrensis* 22 kg, *Tortula ruralis* 1 kg).

1985. May-Jun--Oregon and California: WBA expedition, ~ 100 general samples (25-100 g/sample) of lichens for the NCI antitumor and anti-AIDS screening; received assistance in identifications from Lawrence Pike for collections from Oregon, Mason Hale for Parmeliaceae, Clifford Wetmore for Collemataceae, and Richard Harris for *Lepraria*. **Jul--Eastern U.S. (Maryland to Florida):**. WBA--- 50 samples of lichens (NCI). **Oct-Nov--West Virginia, Virginia, and Maryland:** WBA re-collections of bryophytes (*Bartramia pomiformis, Eurhynchium pulchellum, Polytrichum ohioense*, 2 kg or 50 kg/sample dried) for isolation of antitumor agents (PU, VPISU).

1984. Aug--Western U.S (Colorado, Utah, Arizona, New Mexico) and Tennessee: WBA expeditions, 17 general samples (100+ g/sample) of lichens for antitumor screening (VPISU); re-collection (1 kg) of a moss (*Anomodon attenuatus*); lichens identified by Mason Hale. **Jul-- Northeastern U.S: (primarily White Mountains in New Hampshire and Maine);** re-collections (10-20 kg/sample) of bryophytes (*Anomodon attenuatus, Dicranum fulvum, Polytrichum pallidisetum, Bazzania trilobata, Diphyscium foliosum, Pleurozium schreberi, Ptilium crista- castrensis)* for antitumor screening (PU, VPISU).

1983. Jul--California: WBA re-collection of a moss (*Plagiomnium venustum*) for isolation of antitumor active plants (Univ. Illinois at Chicago).

1981. Texas, Nevada, California and Oregon. Apr-Jun. USDA re-collections of vascular plants (50 kg/sample)--*Erioneuron pulchellum, Kalanchoe tubiflora, Gutierrezia microcephala,* and *Iris missouriensis*, and 100 kg re-collection of a moss, *Claopodium crispifolium,* for isolation of antitumor agents; 57 general samples (1-2 kg/sample) of selected medicinal plants for antitumor screening (NCI).

1980. US-Wide. USDA--200 (1-2 kg) samples of bryophytes for antitumor screening: Tennessee and North Carolina (The Great Smoky Mountains National Park), and Kentucky (Nov); West Virginia (Oct); Maryland, Virginia, Pennsylvania, New Hampshire and Maine (Sep); California (Jul). USDA---100 general samples (1-2 kg) of vascular plants from California, Arizona, Utah and Nevada for antitumor screening; recollections of vascular plant-*Dirca occidentalis* and mosses--*Claopodium crispifolium* 50 kg and *Plagiomnium venustum* 150 kg (May-Jun) for isolation of antitumor agents (NCI).

1979. Colorado, Nevada, California and Oregon. Sep and Feb-Apr. Two USDA expeditions. Re-collections (50 kg/sample) of *Ipomopsis aggregata* and *Horkelia fusca*; ~125 general samples (1-2 kg/sample) of higher plants and 17 species of bryophytes for anticancer screening (NCI).

1978. Nov-Dec--Texas (Big Bend National Park), Nevada and California: USDA-- ~100 samples (1-2 kg/sample) for NCI antitumor screening (NCI). **Sep--Virginia, North Carolina and Tennessee:** USDA---50 general samples (1-2 kg/sample) for the NCI antitumor screening (NCI). **May-Jun--Texas and California:** USDA reconnaissance of the Chuckawalla Mountains (California) and of southern Texas to locate large stands of *Colubrina californica* and *Colubrina texensis*--1 ton samples were needed from each species to isolate colubrinol, an alternative compound to maytansine which at the time was undergoing clinical studies in the treatment of human cancers; coordinated volunteered assistance from the Boy Scouts of America in the procurement of 250-1000 kg samples; obtained 75 general samples (1-2 kg/sample) for antitumor screening (NCI).

1973. Wisconsin. Jul. USDA ground and aerial reconnaissance of Wisconsin and southern Ontario for *Thalictrum dasycarpum*; to identify sites that might provide a cumulative yield of 4 tons of seed. This was for isolation of thalicarpine, a compound undergoing clinical evaluation for treating cancers in humans. **Arizona and California** Apr-May. USDA--20 re-collections of plants for anticancer screening (NCI).

1967-1972 - California. Obtained ~ 300 samples (.5-1 kg/sample) for antitumor screening (NCI via contract with USDA), during a 4 1/2 month period in southern California, especially in the Mohave and Colorado Deserts, also ~ 50 large samples (50 kg/sample) from northern and southern California; ~ 1,300 collections towards M.A. thesis, and ~1,000 collections for personal research from wilderness areas-- Trinity Alps, Marble Mountains, Yolla Bolly Mountains, and Salmon Scott Mountains.

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Structure, Absolute Stereochemistry, and Synthesis of Conocurvone, a Potent, Novel HIV-Inhibitory Naphthoquinone Trimer from a *Conospermum* sp.[1]

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Contribution from the Laboratory of Drug Discovery Research and Development, Developmental Therapeutics Program, Division of Cancer Treatment, National Cancer Institute, Building 1052, Room 121, Frederick, Maryland 21702-1201

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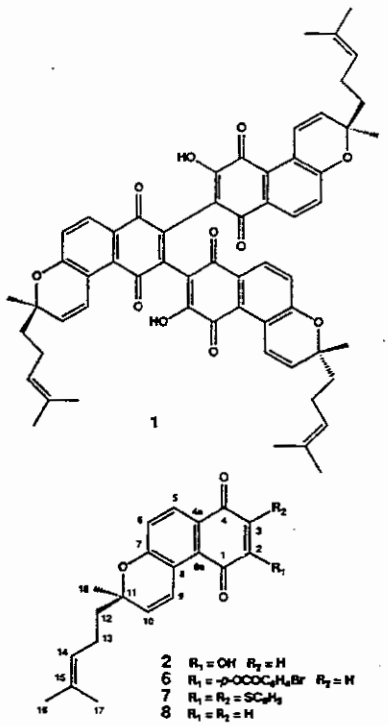
Abstract: Bioassay-guided fractionation provided conocurvone (**1**), a novel trimeric naphthoquinone derivative, as the active anti-HIV constituent of an extract from a *Conospermum* sp. The related naphthoquinone monomer teretifolione B (**2**) also was isolated from a *Conospermum* sp. extract. The absolute stereochemistry of **2** was established by X-ray crystallographic analysis of the *p*-bromobenzoate derivative **6**. Base-catalyzed coupling of 2 equiv of teretifolione B (**2**) with the deoxy derivative **8** provided compound **1**, which was identical in all respects with the natural product. While compound **2** was inactive against HIV, the natural and synthetic conocurvone (**1**) and the synthetic trimeric analog **4** were all active and equipotent, preventing the cytopathic effects and replication of HIV in human T-lymphoblastic cells (CEM-SS) over a concentration range of 0.02–50 μM.

As part of a major new natural products drug discovery and development initiative, the U.S. National Cancer Institute has been screening extracts from diverse terrestrial plants, marine organisms, and microbial sources for anti-HIV or selective cytotoxic properties.[2] The initial focus of the present study was an organic extract from the endemic Australian shrub *Conospermum* sp. (Proteaceae) (Spjut 7139), which inhibited the cytopathic effects of HIV-1 infection in the NCI's primary *in vitro* anti-HIV screen.[2,3] Bioassay-guided fractionation of the extract provided conocurvone (**1**), a novel trimeric naphthoquinone derivative, as the principal anti-HIV constituent. A solvent/solvent partitioning protocol applied to the crude extract concentrated HIV-inhibitory activity in the nonpolar fractions. This material was then subjected to centrifugal countercurrent chromatography (hexane/EtOH/EtOAc/H_2O, 5:4:2:1, ascending mode). Active fractions from the countercurrent chromatograph were pooled and further separated by low-pressure column chromatography on diol-bonded phase packing eluted with increasingly polar mixtures of hexane/EtOAc. Final purification of the anti-HIV constituent conocurvone (**1**) was achieved by HPLC on a phenyl-bonded phase column with CH_3CN/H_2O (17:3, 0.1% HOAc by vol).

During fractionation of a *Conospermum* sp. extract, a related monomeric species that was inactive against HIV was isolated and identified by spectral analysis as teretifolione B (**2**). This compound had previously been identified as one of a series of quinones from *C. teretifolium*.[4,5] While the structure of **2** was originally verified by synthesis, no NMR, UV, or IR data have been available for the compound.[4] Our independent spectrochemical characterization of teretifolione B (**2**) confirmed the structure, and proton-detected heteronuclear correlation experiments (HMQC and HMBC) allowed the complete assignment of all ^1H and ^{13}C NMR resonances.



The ^1H NMR spectrum of conocurvone (**1**) is very complex, with many highly overlapped resonances (Figure 1). In addition, the aromatic proton resonances (Figure 2) are flanked by satellite peaks that apparently integrated for less than one proton. The satellite peaks were originally suspected to arise from a chromatographically inseparable impurity that coeluted with conocurvone (**1**). However, the complexity and chemical shifts of

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(1) Part 11 in the series HIV Inhibitory Natural Products. For part 10, see: McKee, T. C.; Cardellina, J. H., II; Tischler, M.; Snader, K. M.; Boyd, M. R. *Tetrahedron Lett.* **1993**, *34*, 389–392.

(2) Boyd, M. R. *AIDS Etiology, Diagnosis, Treatment and Prevention*; Lippincott: Philadelphia, PA, 1988; pp 305–319.

(3) Weislow, O. S.; Kiser, R.; Fine, D. L.; Shoemaker, R. H.; Bader, J.; Boyd, M. R. *J. Natl. Cancer Inst.* **1989**, *81*, 577–586.

(4) Cannon, J. R.; Joshi, K. R.; McDonald, I. A.; Retallack, R. W.; Sierakowski, A. F.; Wong, L. C. H. *Tetrahedron Lett.* **1975**, 2795–2798.

(5) Thompson, R. H. *Naturally Occurring Quinones* III: *Recent Advances*; Chapman & Hall: London, 1987; p 208.

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 Commonwealth Scientific and Industrial Research Organization (Australia), Central Drug Research Institute (India), National Defense Medical Center (Taiwan), University of Arizona, University of Costa Rica, University of Concepcion (Chile), University of Brazil (Rio de Janeiro), and the University of Hawaii (J. L. Hartwell, pers. comm.).

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[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.

Economic Botany, 39(3), 1985, pp. 266–288

Richard Spjut (BS–1969, MA–1971, Humboldt State University, CA) began his professional career as a graduate student in 1970 by collecting plant samples from California for the National Cancer Institute's (NCI) antitumor screening program through contracts with the USDA Agricultural Research Service (ARS), and by completion of a thesis on mosses of the Marble Mountain Wilderness. He was subsequently hired by the ARS in May 1972 where he continued the NCI work under an ARS-NCI cooperative agreement, collecting in Western Australia, Tasmania, Ghana, Kenya, Tanzania, Zambia, Mexico, and United States. His plant collection strategies have focused on plants in mediterranean and semi-desert scrub based on his studies of NCI screening data that suggested a greater chance of finding novel compounds in roots of plants from these areas; he published several reviews *Economic Botany*, 1985, 1986. One of his more successful expeditions was in Western Australia (1981): 758 samples (1.5–2.5 kg each) were collected during a six-week period, which led to isolation of a promising new anti-HIV drug— conocurvone—from the root of *Conospermum* sp. (Proteaceae). When NCI terminated their agreement with ARS in 1982, he was re-assigned to other ARS activities, one of which led to this paper; however, he continues to collect samples for drug-discovery programs through World Botanical Associates. Additionally, as a collaborator of the Smithsonian Institution since 1986, he has undertaken a taxonomic revision of the genus *Taxus*, species which contain the anticancer drug taxol, and the lichen genus *Niebla* of Baja California.

From Back page in Systematic Treatment of Fruit Types.