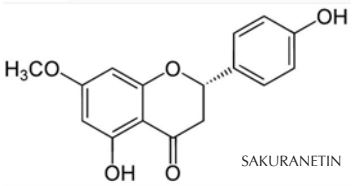
Photo (R) Paul Gipe; (L) R. Spjut

Eriodictyon californicum (**California yerba santa**), shrubs (left) near McCloud River, Shasta Lake – May 12, 2009. Flowers close-up (right) Old Kern Canyon Rd. – 27 Apr. 2013.
Leaves have been used by native American tribes for treating colds, coughs, fevers, rheumatism, asthma, pulmonary, dermatological and stomach problems.[1] Species one of six among 97 samples (WBA-656, SPJ-10440) I recommended on a shipping list for antiviral and antitumor screening in addition to the chemopreventive screening at the Ohio State University School of Pharmacy. Twelve flavonoids found to inhibit metabolism of the carcinogen benzo[a]pyrene by hamster embryo cells in tissue culture.[2] The flavanone, sakuranetin, inhibited the response to saccharin by more than 50%.[3] Additionally, sakuranetin has shown antiproliferative activity against human cancer cell lines typical for B16BL6 melanoma, esophageal squamous cell carcinoma (ESCC) and colon cancer (Colo 320), and reported to have "*antiviral activity towards human rhinovirus 3 and influenza B virus.*"[4]



President's Message:
Antiviral Plants: Chemical in Kern County Plant Could Treat COVID-19?

by Rich Spjut

MY CAREER HAS BEEN LARGELY DEVOTED to discovery of new chemotherapeutic agents from plants including bryophytes, and also from lichens, cyanobacteria and marine algae. The collection strategy has been based on taxonomy, recognizing that the genus is the lowest level of chemo-taxonmic diversity,[5] while reviewing plants used in traditional medicine.[6] Most samples I collected were screened for antitumor, antibiotic[7], antiviral, antiprotozoal[8], and chemopreventive agents;[9] fewer for isolation and identification of carotenoids[10] and endophytes and **endolichenic** microbes[11] that produce the antitumor active compounds. An example of an endolichenic fungus is *Penicillium aurantiacobrunneum,* cultured from the lichen *Niebla homalea,* that led to discovery of four new mycotoxins related to citreoviridin, one of which showed selective cytotoxicity towards ovarian and breast cell line assays.[12] Higher plants from which anticancer compounds have been isolated often harbor endophytic fungi and bacteria that produce the same or similar compounds.[13] Moreover, antitumor activity in many moss species appears related to their microbial associations.[14,15,16] Nearly half ("49%") of all chemotherapeutic agents used in medical prescriptions or prescribed treatments can be attributed directly or indirectly to discoveries from natural products; the active chemicals are "small molecules," in contrast to large molecules (nucleic acids, proteins, polysaccharides).[17]

> Nearly half... of all chemo-therapeutic agents used in medical prescriptions or ... treatments can be attributed directly or indirectly to discoveries from natural products.

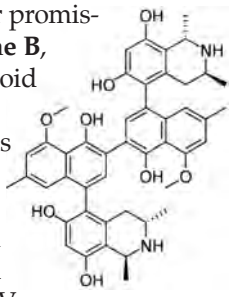
Recently, our chapter activities, like those of other CNPS chapters, have been curtailed by the spread of a coronavirus, SARS-CoV-2 — that causes COVID-19 — classified in the genus *Betacoronavirus*, one of six genera in the family Coronaviridae. More than 30 families of viruses are recognized to infect humans and other vertebrates as outlined on the website "viral zone,"[18] which classifies viruses by nomenclatural taxonomy, and by other criteria such as their host — including invertebrates, plants, fungi, eukaryotic microorganisms, bacteria and archaea. From my experience, I wondered to what extent antiviral compounds have been isolated from plants and lichens and advanced to clinical trials. A brief review follows.

One can easily find online reports of antiviral activity in plants and lichens; for example, 795 plant species reportedly used for treating HIV[19] included 23 species — mostly nonnative — found in Kern County.[20] But it must be kept in mind that very few reach human clinical trials; for example, ~10% of 270 lichen species that I collected for the **National Cancer Institute** (**NCI**) from the United States and Mexico during 1986 – 1992 were "active" in HIV assays due to sensitivity to polysaccharides[21] whose immunomodulating effects are already generally known in metabolism and food but nonetheless of pharmacological interest.[22,23] On the other hand,

many of 344 medicinal plant compounds reported to show activity in HIV, herpes and other viral assays[24] have been clinically evaluated in cancer chemotherapy.

Antiviral plant compound - MICHELLAMINE B

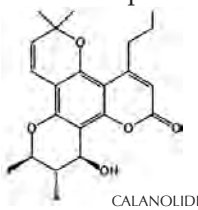
During 1987–1996, the NCI screened more than 60,000 plant extracts in HIV assays.[25] Of four promising discoveries, one was **michellamine B**, a naphthyl-isoquinoline dimeric alkaloid isolated from leaves of a rare tropical rain forest woody vine (liana) that was later discovered to be a species new to science, ***Anscistrocladus korupensis*** (Ancistrocladaceae), found only in southwest Cameroon. The compound was active against many strains of HIV-1 and anti-HIV-2; however, in animal preclinical trials it had a very narrow therapeutic dosage between efficacy and toxicity.[26]



MICHELLAMINE B

Antiviral plant compound - CALANOLIDE A

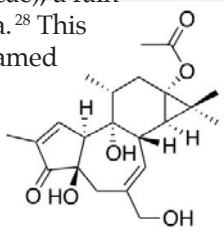
Another NCI anti-HIV discovery, (+) **calanolide A**, a pyranocoumarin isolated from the latex of rare tropical forest tree, ***Calophyllum lanigerum*** (Calophyllaceae), in Sarawak, Malaysia, advanced to HIV Phase I clinical trials with promising results, but development "delayed due to its low therapeutic index (range: 16–279), non-ideal antiviral activity, and the complexity of its extraction from plants;" however, a calanolide derivative is being investigated in animal studies for the treatment of HIV in combination therapy.[27]



CALANOLIDE A

Antiviral plant compound - PROSTRATIN

A third anti-HIV compound of interest is **prostratin** — an unusual phorbol ester (tetracyclic diterpene) in not being tumorogenic as opposed to many phorbol esters in Euphorbiaceae and Thymelaeaceae that cause tumors. Prostratin was isolated from the stembark of ***Homalanthus nutans*** (Euphorbiaceae), a rain forest tree in central Polynesia, Samoa.[28] This species — originally described and named *Croton nutans* by **J.G.A. Forester** in 1786 from a specimen he and his father collected on Tonga — has since been recognized to occur in Fiji, Society Islands, New Caledonia, and New Hebrides,[29] distinguished from the closely related and more widely distributed *H. populneus*.[30] In Fiji the local inhabitants prepare an aqueous infusion of the leaves and drink it for stomach troubles, and take a fruit decoction for relief of urination problems (*ibid.*). Similar uses have been noted in Samoa. An expert on the vegetation, flora and ethnobotany of Samoa, **W. Arthur Whistler**, who was born near Death Valley, recently died from coronavirus, apparently infected while visiting in the state of Washington. Prostratin is in clinical trials in which its efficacy has been compared with that of a polyketide, bryostatin, discovered in a bryozoan, *Bugula neritina*,[31] the compound also in clinical trials for treating cancer.[32]

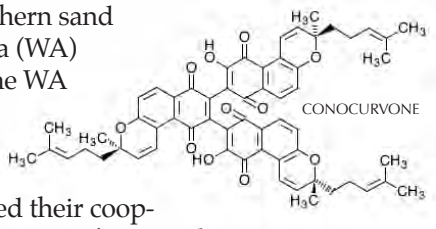


PROSTRATIN

Above: Aerial view of smokebush species, *Conospermum stoechadis* ssp. *stoechadis* – Aug 1992. **Below:** ground view, northern sand plains between Perth and Geraldton, Western Australia. This species appears to be the most conspicuous and common of the 20 smokebush species in section *Foliosa*, but not known to contain conocurvone. A survey was conducted by Richard Spjut and Ross Smith of World Botanical Associates (WBA) under a NIH-NCI Master Agreement Award to identify where best to collect *Conospermum* species for anti-HIV screening of concurvone.

Antiviral plant compound - CONOCURVONE

A fourth NCI anti-HIV compound, **conocurvone**, is a naphthoquinone, isolated from the root of a smokebush species, ***Conospermum incurvum*** (Proteaceae), that I collected on the northern sand plains of Western Australia (WA) during Aug-Sep 1981.[33] The WA field work came to a sudden halt when I was informed — that after 20 years — the NCI terminated their cooperative agreement with the **USDA/ARS** at the start of the new fiscal year. At that moment it was uncertain whether any of the 758 samples would be screened for their intended purpose — as new anticancer drugs — especially since no money appeared available to ship the samples to the US. The curator of the WA herbari-



CONOCURVONE

Smokebush AIDS drug pact reached

By Brendan Nicholson

CLINICAL trials of an AIDS drug derived from a chemical found in WA's native smokebush might be carried out experimenting with new approaches to the smokebush chemical, conocurvone.

Even if the research was unsuccessful, the effort could produce invaluable information about the AIDS virus.

Top: *Conospermum incurvum*, near Perth, Western Australia, photo documented by herbarium specimen, SPJ 12464. Root contains conocurvone. **Bottom:** News article from a WA newspaper regarding agreement between the NCI and Australian Medical Research and Development (AMRAD) to pursue research on conocurvone HIV activity.

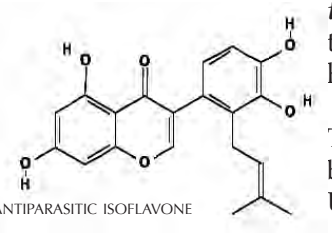
um expressed his disappointment, while also conveying that they could not use or store the samples. It was further disappointing in that I had had to amend my license to allow for the collection of root samples.

However, I successfully lobbied to get the samples shipped to my office facility in Maryland at the **Beltsville Agricultural Research Center.** After accounting for their identification and dry weight, they were shipped to the extraction laboratory in Wisconsin from where many went to **Purdue University** and to **Research Triangle Institute** (**RTI**) in North Carolina. **Monroe Wall** at RTI, who isolated the anticancer drug taxol from the Pacific yew, *Taxus brevifolia*, based on original activity in the KB assay,[34] reported KB activity in nine WA species.[35] The KB test results for 181 of 340 samples at Purdue University were sent to me in 1986 by **Thomas McCloud**, a graduate student of **Dr. John Cassady**; these results were published on the WBA website in 2014.[36] Although *Conospermum* was not among the samples screened against KB, the test results indicated the significance for collecting root; 15 of 24 (63%) of the active species in WA woody plants had activity only in root.

The WA samples, among many others at Purdue University, were retrieved and brought to the **NCI Natural Products Repository** in Frederick, MD by Dr. McCloud for anti-HIV screening during the time when the NCI was developing their antitumor 60-cell-line assays. Discovery of anti-HIV activity in the smokebush led me to visit WA in 1991 for recollections, encouraged by WA authorities. Additional material needed in 1992 led to a partnership, **World Botanical Associates-Australia**. Agreements were initiated with WA government authorities, including a NCI letter of Collection, and proposals were sought with Kings Park for cultivation of smokebush in tissue cultures. Several hundred species also were collected for preliminary screening of novel bio-active agents. But only a temporary agreement could be reached. After I left WA, conocurvone continued to be pharmacologically evaluated in Australia, while I have not seen reports on its advancement in HIV screening. Nevertheless, concurvone has emerged as "one of the most notable members" of the naphthoquinone class of compounds that — in its synthesis — has led to a "series of novel dimeric naphthoquinones" that show "selective cytotoxic to human acute myeloid leukemia (AML), breast and prostate cancer cell lines."[37] A "relatively simple naphthoquinone, ß-lapachol" "obtained from the bark of the South American lapacho tree, (*Handroanthus impetiginosus*), has been in phase II clinical trials in the US for advanced solid tumors.[38]

New leads gleaned from databases...

As field exploration of plants continue to find leads to new therapeutic drugs, chemists using computer algorithms search "ligand databases"[39] on the genomic, structural, reactive and binding properties of known molecules; in essence, they virtually screen compounds for new drug therapies, ranking the results as one might find from a search in Google looking for specific information using key words. Recently, chemists at universities in China and Saudi Arabia – in collaboration – reported screening 32,297 "potential antiviral phytochemicals" for treating COVID-19 by targeting the enzyme that controls coronavirus replication as seen in the severe acute respiratory syndrome coronavirus (SARS-CoV), which has a very similar genome sequence to SARS-CoV-2 ("96.08%"). Their highest ranking compound was a **7-Hydroxy isoflavone** — 5,7,3′,4′-tetrahydroxy-2′-(3,3-dimethylallyl) — isolated from the root of ***Psorothamnus arborescens*** var. ***minutifolius*** (**Mojave indigo bush**), the species and variety found here in Kern County.[40]



ANTIVIRAL& ANTIPARASITIC ISOFLAVONE

The isoflavone was discovered by chemists at the Ohio State University–School of Phar-

macy as a result of screening plants for activity against leishmaniasis, a disease caused by an infected sand fly bite, inoculating the victim with the protozoan (*Leishmania*).[41] The isolation of the compound was guided by fractionation of the active extracts prepared from 670 g of dried root (WBA-4841-13) of *P. arborescens* var. *minutifolius* collected near the Kern County line (Nine-Mile Canyon) "in desert-chaparral transitional vegetation by **Dr. Richard Spjut** (WBA 4841-13, SPJ-15357), **World Botanical Associates** (Bakersfield, CA)." **Dr. Salem** in her Ph.D. dissertation (2005, Ohio State University) had reported the isoflavone and other flavonoids, and also those reported earlier from the aerial parts of *P. polydenius*. She concluded — in her abstract: "From the results [of screening 174 biodiversity samples collected and 149 extracts from selected plants in China used in Chinese medicine], the plant genus *Psorothamnus* was identified as a promising source of potential new antiparasitic compounds."

Of additional interest is that the percentage of antiparasitic actives was higher in my selection of samples (19/174, 11%) than in the selection from plants used in Chinese medicine (10/149, 6.7%), and that the sample of the invasive white horehound (*Marrubium vulgare*), I collected in Nevada (leaf-flower-fruit, 65 g) showed the "same activity" as the sample from China (native). As reported by Dr. Salem, my collection strategy focused "on samples not generally collected for the National Cancer Institute (NCI) based on the NCI requirement of 500 g" with emphasis on "root and stem bark from shrubs, small annual herbs, and flower and fruit parts of all plant species," samples each weighing "30–100 g."

The WBA samples Dr. Salem reported on were collected from San Diego County to southern Oregon and in western Nevada during June 14 to July 10, 1998. An example of a small annual is *Mimulus bigelovii*; a 45 g sample (WBA-3664) of the entire herb led to discovery of selective antileishmanial active compounds in the species. This was followed by two recollections, one in 2003 (WBA 4840-11, 130 g) and another in 2005 from limestone talus slopes west of Sandy Valley NV in the Mesquite Mts. in California along Kingston Rd near the state line; active C-geranyl flavonoids were isolated from 1.1 kg (dried) of the whole plant (WBA-5257-11, SPJ-15900).[42]

Environmental variations

Finally, it should be recognized that plants contain many secondary metabolites in different parts of the plant in various concentrations, and the species itself may not always produce the active compounds in all environments. An example is **white corn lily**, *Veratrum californicum* (Melanthiaceae). I conducted surveys in the western US in 2004–2011. A semi-synthetic derivative of the active alkaloid, **cyclopamine**, depended on the plant source for synthesizing the derivative; the alkaloid concentrated in the root-rhizome-bulb. Plants that produced the alkaloid were primarily found in a narrow geographic range of the species, and only in specific types of vegetation.[43] Even among clones, the yield in cyclopamine varied, suggesting the active compound further depended on mycorrhizal association. ✿

Mojave Indigo Bush (*Psorothamnus arborescens* var. *minutifolius).* Highest-ranked plant worldwide for containing a chemical in the root — **isoflavone**, 5,7,3′,4′-tetrahydroxy-2′-(3,3-dimethylallyl) — that might prove useful for treating COVID-19. Shrub (above) and close-up flower (below) taken in western Mojave Desert, Short Canyon, Kern County – March 2016 and April 2019. Related species, *P. polydenius*, used by the Paiute and Shoshoni for colds, measles, smallpox, pneumonia, whooping cough, and tuberculosis;[1] its active flavonoids were reported 2004 in the Journal of Natural Products (68: 108–111) by Drs. M. M. Salem and K. A Werbovetz.