

Plant Secondary Metabolites as Potential Anticancer Agents and Cancer Chemopreventives

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There is considerable interest in the screening of plant and other natural product extracts in modern drug discovery programs, since structurally novel chemotypes with potent and selective biological activity may be obtained [1-4]. A consideration of biological activity in addition to the isolation and structure elucidation stages in a phytochemical investigation may add a great deal to the overall scientific significance of the work. Phytochemists may gain considerable information by using panels of simple bioassays and/or more specialized *in vitro* bioassays to follow each step of a purification procedure [3]. In the following paragraphs, recent examples of bioactive compounds obtained in the author's laboratory in projects directed towards the search for novel anticancer agents and cancer chemopreventives from higher plants will be presented.

In the United States in 1999, it is estimated that over 1.2 million persons will be diagnosed with invasive forms of cancer, and over 1,500 people will die as a result of cancer each day [5]. Among many recent advances in cancer chemotherapy, plant natural products have played an important role in contributing to the arsenal of the approximately 60 cancer chemotherapeutic drugs on the market. For instance, in the United States, there are now four structural classes of plant anticancer agents available, constituted by the *Catharanthus* (Vinca) alkaloids (vinblastine, vincristine, vinorelbine), the epipodophyllotoxins (etoposide, etoposide phosphate, teniposide), the taxanes (paclitaxel and docetaxel), and the camptothecin derivatives (irinotecan and topotecan) [6]. Several other plant-derived compounds are currently in preclinical and clinical trials [6,7].

As part of a National Cooperative Natural Products Drug Discovery Group (NCNPDDG) research project funded by the United States National Cancer Institute (1995-2000), our collaborative team at the College of Pharmacy, University of Illinois at Chicago (Chicago, Illinois), and Research Triangle Institute (Research Triangle Park, North Carolina), and Bristol-Myers Squibb (Princeton, New Jersey) is evaluating about 400 plant samples per year, with the aim of discovering and evaluating novel plant-derived anticancer agents. During the funding period 1990-1995, the industrial partner was Glaxo Wellcome Medicines Research Centre (Sevenage, U.K.), and past progress made in the project has been reviewed [8]. Since 1995, the primary plant samples have been collected in the Dominican Republic, Peru, and Indonesia. Plant recollections have taken place mainly in Thailand and Zimbabwe in

recent years. Our funding agency requires that we obtain permission through formal written agreements to acquire plants for research. For each plant acquisition, a non-polar extract is prepared and screened against batteries of cultured human cancer cells and panels of mechanism-based assays. An LC-MS dereplication procedure has been developed to attempt to avoid the re-isolation of common classes of known cytotoxic compounds [9]. As a result of bioactivity-guided fractionation on selected plant leads, well over 100 active compounds have been isolated and structurally characterized in the project to date. Many of these of novel structure and several have been further evaluated in secondary *in vitro* bioassays and *in vivo* assays. Examples of active compounds obtained in this project include 1*H*-cyclopenta[*b*]benzofuran derivatives from *Aglaia elliptica* (Meliaceae) [10], phenanthrene derivatives from *Domohinea perrieri* (Euphorbiaceae) [11], resveratrol tetramers from *Vatica diospyroides* (Dipterocarpaceae) [12], and sesquiterpene lactones from *Ratidiba columnifera* (Asteraceae) [13].

Plant secondary metabolites also show promise for the cancer chemoprevention, which has been defined as “the use of non-cytotoxic nutrients or pharmacological agents to enhance physiological mechanisms that protect the organism against mutant clones of malignant cells” [14]. There has been considerable prior work on the cancer chemopreventive effects of extracts and purified constituents of certain culinary herbs, fruits, spices, teas, and vegetables, which have shown the ability to inhibit the development of cancer in laboratory animal models [15,16]. Clinical trials as cancer chemopreventive agents under the auspices of the United States National Cancer Institute are planned for plant products such as curcumin, ellagic acid, and phenethyl isothiocyanate [17].

In our project on cancer chemopreventive agents from plants, novel compounds are again isolated from plant extracts by activity-guided fractionation techniques, although a different panel of bioassays is employed in comparison to the anticancer agent project described above. The project is funded by the National Cancer Institute, through the Program Project mechanism, and all of the work is performed at the University of Illinois at Chicago [18,19]. The plant material is constituted both by food plants and by species collected in the field, and an organic-soluble extract is obtained from each milled plant part. Preliminary biological evaluation is carried out using a panel of about ten short-term *in vitro* bioassays, with some being relevant to each of the initiation, promotion, or progression stages of carcinogenesis [20]. Biological follow up occurs using a mouse mammary organ culture model [21], and, in a very few selected cases, evaluation in a two-stage mouse skin or rat mammary carcinogenesis model [22]. Once again, in the project to date over 100 active compounds have been obtained, of which some have been subjected to *in vivo* biological characterization. Examples of active compounds obtained in this project include a number of antimutagenic alkaloids, coumarins, and flavonoids from the seeds of *Casimiroa edulis* (Rutaceae) [23], withanolides from *Physalis philadelphica* (Solanaceae) (“tomatillos”) with the ability to induce levels of the enzyme quinone reductase [24], antioxidant flavonoids from *Chorizanthe diffusa* (Polygonaceae) [25], and some steroidal alkaloids from *Pachysandra procumbens* (Buxaceae), which showed significant activity in an antiestrogen-binding assay [26].

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