

Novel strategies for the discovery of plant-derived anticancer agents*

A. Douglas Kinghorn¹, Norman R. Farnsworth¹, D. Doel Soejarto¹
Geoffrey A. Cordell^{1†}, John M. Pezzuto¹, George O. Udeani¹,
Mansukh C. Wani², Monroe E. Wall², Hernán A. Navarro²,
Rob A. Kramer³, Ana T. Menendez³, Craig R. Fairchild³,
Kate E. Lane³, Salvatore Forenza⁴, Dolotrai M. Vyas⁴,
Kin S. Lam⁴ and Yue-Zhong Shu⁴

¹College of Pharmacy, University of Illinois at Chicago, Chicago, IL 60612, USA;

²Research Triangle Institute, Research Triangle Park, NC 27709, USA;

³Bristol-Myers Squibb, Pharmaceutical Research Institute, PO Box 4000, Princeton, NJ 08543, USA;

⁴Bristol-Myers Squibb, Pharmaceutical Research Institute, Research Parkway, Wallingford, CT 06492, USA

Abstract: Several plant secondary metabolites or their semisynthetic derivatives are used clinically as cancer chemotherapeutic agents. In this review, a multidisciplinary collaborative research program focused on the discovery of novel anticancer agents from tropical rainforest plants is described. This team approach has integrated aspects of botany, biology, and chemistry. Examples are presented of active compounds isolated and biologically evaluated in recent work in this project.

INTRODUCTION

In the United States in 1999, over 1500 people are expected to die of cancer each day, representing an estimated total mortality rate of about 560 000. More than twice as many persons than this will be diagnosed with invasive cancer, but, overall, a slight decline in cancer incidence rates has been observed recently in the USA [1]. However, there is still cause for considerable concern, because on a world-wide basis, the latest figures for the year 1990 show that the rate of growth in cancer cases (2.1% per year) is superseding that of the overall population increase (1.7%/year) [2]. Among many recent advances in cancer chemotherapy, plant natural products play an important role in having contributed considerably to the approximately 60 available cancer chemotherapeutic drugs. There are now four structural classes of plant-derived anti-cancer agents on the market in the USA, constituted by the *Catharanthus* (Vinca) alkaloids (vinblastine, vincristine and vinorelbine), the epipodophyllotoxins [etoposide, etopophos (etoposide phosphate), and teniposide], the taxanes (paclitaxel and docetaxel), and the camptothecin derivatives (topotecan and irinotecan), with several of these being approved for therapy only in the last few years [3–7]. A number of other secondary metabolites and their derivatives of plant origin, as well as natural products of marine and microbial origin, are currently in preclinical and clinical trials as potential anticancer agents [3–7]. Accordingly, there is considerable interest in the discovery of additional novel natural products and their semisynthetic analogs as potential cancer chemotherapeutic drugs.

*Plenary lecture presented at the 2nd International Conference on Biodiversity, Belo Horizonte, Brazil, 11–15 July 1999, pp. 1611–1690.

†Corresponding author: E-mail: cordell@uic.edu

In 1989, the National Cancer Institute (NCI), Bethesda, Maryland, established the National Cooperative Natural Product Drug Discovery Group (NCNPDDG) grant mechanism to 'discover and evaluate new entities from natural sources for the treatment and cure of cancer' [8]. As one of several consortial groups funded through this mechanism, scientific teams from the College of Pharmacy, University of Illinois at Chicago, Chicago, Illinois and the Research Triangle Institute, Research Triangle Park, North Carolina have collaborated for nearly a decade in a NCNPDDG project directed towards the discovery and biological evaluation of novel anticancer agents from tropical rainforest plants. Two pharmaceutical companies have been industrial partners in this joint venture. Initially, Glaxo Wellcome Medicines Research Centre, Stevenage, UK (1990–1995) was involved, while currently Bristol-Myers Squibb, Pharmaceutical Research Institute, Princeton, New Jersey and Wallingford, Connecticut (1995–2000) is the industrial collaborator in this drug discovery program. The overall project organization and examples of promising compounds obtained to date have appeared in previous reviews and book chapters [9–11].

PLANT COLLECTIONS

Since the beginning of the second 5-year phase of our collaborative project in 1995, about 400 plant parts are collected each year, primarily from tropical rainforest areas, through the cooperation of a network of botanist collaborators. While tropical rainforests offer considerable biodiversity, they occupy only about 8% of the surface area of the terrestrial regions of the earth, and are being destroyed at an alarming rate [12]. In our study, priority is given to species endemic to a particular country, and up to four anatomical parts of each plant may be collected. Taxonomic authentications are determined primarily in the country where the plant is collected, and several sets of voucher specimens are deposited in various herbaria [9–11]. The University of Illinois at Chicago campus has been a leader in establishing policies and procedures for the agreements necessary to collect and/or receive plants, and to provide for appropriate compensation, recollection, crop development and royalty distribution commitments, as well as for initial payment for the samples [13,14]. In some instances, we have also provided training programs or conducted symposia for local personnel. We are continuously working with local governments and in-country organizations to assure that our access meets local requirements and contemporary ethical value systems. Strategic decisions have been made not to collect in certain countries after negotiations to collaborate and provide reasonable compensation packages were unsuccessful. The National Cancer Institute requires our National Cooperative Natural Product Drug Discovery Group project team to obtain permission to acquire plants through formal written agreements with host countries, before any funding for collections can be authorized.

BIOLOGICAL EVALUATION

In the project to date, we have tried to maximize our prospects of successfully meeting the project goals by screening initial plant extracts through batteries of cell-based and mechanism-based bioassays relevant to cancer, and the basic approach has been described in some detail previously [9–11]. Diverse *in vitro* bioassays are used to monitor activity-guided fractionation. In performing this type of research endeavor it is important that the level of potency found in the bioassay selected is not lost during fractionation, and that the biological activity recorded is concentrated over a reasonable number of chromatographic column cuts. Once isolated in pure form, active compounds are evaluated in all of the *in vitro* bioassays available to the project, and a preliminary notion of the compound's mechanism of action may also be determined. Compounds may then be selected for an *in vivo* evaluation, using an established tumor model, dependent on a number of factors, including potency and selectivity in the preliminary screening, structural novelty, and substance availability. Examples of some of the assays used in the project to date are provided subsequently.

PHYTOCHEMICAL PROCEDURES

A useful protocol has been developed for the initial extraction of plant extracts, involving the partitioning of a methanol extract into chloroform. Once each chloroform extract is washed with 1% sodium chloride, it is relatively free from plant polyphenols ('vegetable tannins'), which tend to interfere

with enzyme-inhibition assays [15]. Bioactive compounds are isolated from plant extracts by activity-guided fractionation, using standard methods of chromatographic separation and spectroscopic characterization [9–11]. Not surprisingly perhaps, new compounds tend to occur in smaller yields than analogs of known structure. It is quite normal for the isolation process to afford a series of structurally related active and inactive analogs, so that in effect a preliminary structure-activity relationship study is performed. As the project has matured, the need to prioritize active extracts for fractionation has become more pressing, and this has been resolved by developing a dereplication procedure.

Dereplication of mixtures of natural products in order to enhance the discovery of new active natural products for further development has been an ongoing effort for at least 40 years [16]. Previous efforts focused on either chemical approaches based on chromatographic or UV spectral properties, or a particular biological profile based on the analysis of the results from several bioassay systems. A significant limitation of these approaches is that they do not interrelate chemical and biological information. One improvement has been the development of bioautographic procedures which correlate biological activities with chromatographic profiles. However, these processes do not permit critical decision-making for an active fraction based on whether the active isolate(s) is likely to be new or known. Elsewhere we have provided the background [16] and some examples [17,18] of our efforts in this area. This dereplication technique is a routine procedure in our laboratory for all active extracts, and operates as follows [16,18]. The active extract at a defined concentration is chromatographed using a standard linear gradient elution system. After passage through a UV detector set at 280 nm, the stream is split 1:50 and the smaller stream mixed with an acid or base-modified post-column solvent prior to introduction into the mass spectrometer. The larger stream is recovered in 96-well plates and evaluated against the bioassay in which the original extract displayed activity. After the bioassay is completed, the masses associated with the biologically active time range(s) are determined from either the positive or negative total ion chromatogram. These masses are then compared with literature data regarding active compounds of that mass, and the compounds of that mass isolated from that plant (or genus of plants). If these data sets combine to indicate the high probability of a known active compound in the extract, the extract is given a very low priority for fractionation. If, on the other hand, the active region of the chromatogram is not associated with any known active metabolite, the extract is of high priority for further studies. An added advantage for the purposes of fractionation is that both the chromatographic region and the mass(es) of the active principle are already established. This procedure has been put to effective use in avoiding unnecessary activity-guided fractionation, because previously known cytotoxic compounds have been identified. Early examples from our NCPDDG acquisitions include some highly functionalized coumarins from *Mesua ferrea* L. (Guttiferae), several iridoids from *Allamandra blanchettii* A. DC. (Apocynaceae), and a number of cyclic peptides from *Rubia cordifolia* L. (Rubiaceae) [16].

An aspect of major importance in this natural product drug discovery project is the periodic need to obtain larger amounts of active compounds of particular promise for more extensive biological testing. This normally involves the need for a recollection of the plant material, which should be performed in the country from which the plant was originally obtained, under conditions as close as possible to the initial collection (e.g. same geographical location, same plant part, same time of year).

RECENT STUDIES

The structures of a number of active compounds isolated and characterized in our recent work on plant anti-cancer agents are shown in Fig. 1 (1–31), and progress on the bioactive constituents of nine of these plants is described in the following paragraphs.

Three *ent*-kaurene diterpenoids have been isolated as cytotoxic constituents of the root bark of *Parinari curatellifolia* Benth. (Chrysobalanaceae), collected in Zimbabwe. These are the known compound, 15-oxozoapatlin (1), and the novel analogs, 13-methoxy-15-oxozoapatlin (2) and 13-hydroxy-15-oxozoapatlin (3). These compounds were broadly cytotoxic when tested in the human tumor cell panel at the College of Pharmacy, University of Illinois (UIC), with the most potent cytotoxic activity being observed in each case in the A431 human epidermoid carcinoma cell line (0.3–0.6 μM in each case) [19]. Since it was obtained in reasonably large quantity, and because of its structural novelty and cytotoxic potency, compound 2 was subjected to a mechanistic investigation. It was found to react with

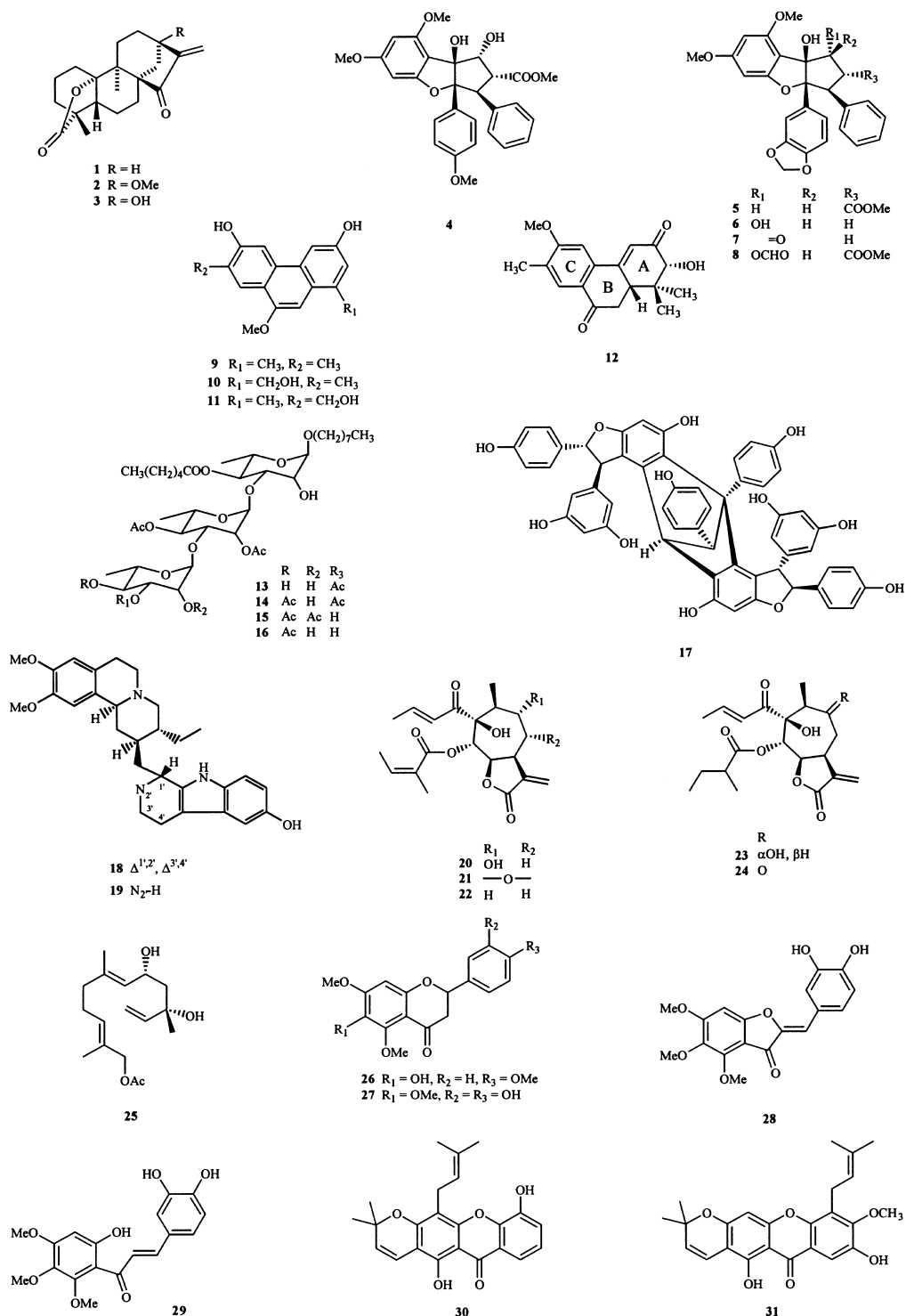


Fig. 1 Structures of bioactive compounds isolated from plants.

the nucleophiles L-cysteine and β-mercaptoethanol, although it did not react with either DNA or guanosine. The effects of this compound were studied on the growth of human ZR-75-1 breast cancer cells, and it was determined that the biosynthesis of DNA, RNA, and protein was reduced in treated cells, and that accumulation at the G₂/M phase of the cell cycle was seen. It was concluded that the cytotoxic activity of 13-methoxy-15-oxozoapatlin (**2**) is mediated in part by means of a Michael-type addition with

a sulfhydryl-containing protein or other cellular component, which results in the blockage of cell-cycle progression [19]. Compound **2** was selected for testing *in vivo* against a KB human epidermoid cancer cell model, implanted subcutaneously, although it was not active at its maximum tolerated dose [19]. However, on the basis of successful preliminary evaluations in the National Cancer Institute 60-cell line tumor panel [20], and an *in vivo* hollow fiber assay [21], 15-oxozaopatlin (**1**) has been selected for future murine xenograft testing.

From *Aglaia elliptica* Bl. (Meliaceae), a tropical rainforest tree obtained from Thailand, the known cyclopenta[*b*]benzofuran, methyl rocaglate (**4**) and four novel analogs (**5–8**) were isolated and structurally characterized [22]. Compound **8** was found to possess an unusual formyl ester substituent. All of these compounds exhibited potent and broad cytotoxicity against a panel of human cell lines, and compound **5** was selected for follow-up biological and mechanistic studies [22,23]. After 24 or 32 h this substance induced accumulation at the G₁/G₀ phase of the cell cycle of cultured Lu1 human lung cancer cells, with normal cell-cycle dynamics observed subsequently at later time periods. During the course of wash-out experiments, colony formation was not reduced, even though cell proliferation was observed in a normal manner. Compound **5** markedly reduced protein synthesis, although it had no effect on nucleic acid synthesis at a much higher concentration level. Accordingly, it was concluded that this compound acts as a cytostatic agent [23]. In a preliminary study on the antitumor potential of **5** on athymic nude mice implanted subcutaneously with BC1 human breast cancer cells, tumor growth was inhibited by treatment with a dose of 10 mg/kg body weight administered intraperitoneally three times a week. This effect lasted for some 23 days, after which tumor growth paralleled that of a control group [23]. Additional biological evaluation of compound **5** is presently being undertaken at the National Cancer Institute.

From the Madagascan plant, *Domohinea perrieri* Leandri (Euphorbiaceae), four new bioactive compounds were isolated, represented by three phenanthrene derivatives (**9–11**) as well as the hexahydrophenanthrene derivative, domohinone (**12**), whose structure and stereochemistry were confirmed by single-crystal X-ray crystallography [24]. Of these compounds, only compounds **9** and **10** were significantly cytotoxic against the human tumor cell panel. However, all four compounds were active in an assay designed to determine bleomycin-mediated DNA strand-scission activity [25], with compounds **9–11** being more potent in this regard than compound **12** [24]. Compounds **9–12** were rated as representing 1.21, 1.08, 1.10, and 0.91 'bleomycin units', respectively, with **9–11** being about 800-fold less potent in the DNA strand-scission assay than bleomycin [24].

Members of an unusual group of acylated oligorhamnosides were obtained as cytotoxic constituents of the stems of the Thai species *Mezzetia leptopoda* (Hook. f. & Thomas) Oliver (Annonaceae). The first of these, compound **13**, was obtained as a novel analog in the mezzetiaside series, and was assigned the trivial name mezzetiaside 8. This was obtained with three known compounds, mezzetiasides 2–4 (**14–16**), as well as additional analogs based on only two rhamnose units, whose structures are not shown. Compounds **13–16** were found to be weakly active as cytotoxic agents, and all showed some selectivity for the Col2 human colon cancer cell line (*ED*₅₀ values of 4.3–8.2 μg/mL) [26].

The stems of the Thai plant *Vatica diospyroides* Sym. (Dipterocarpaceae) afforded an interesting oligostilbenoid as a cytotoxic constituent. This compound, vatdiospyridol (**17**), was assigned as a resveratrol tetramer after extensive analysis of the COSY, HMQC and HMBC NMR spectra, of both the parent compound and a permethylated derivative, and the stereochemistry was postulated using a combination of NOESY NMR data interpretation and energy-minimized molecular modeling. Compound **17** was found to be significantly cytotoxic against human oral epidermoid (KB), colon cancer (Col2), and breast cancer (BC1) cell lines, and is the first resveratrol tetramer to be reported to exhibit cytotoxicity against cancer cells [27]. It is of interest to note that in our investigation on *V. diospyroides* (E)-resveratrol 3-*O*-β-D-glucopyranoside was isolated, providing some circumstantial evidence that the tetrastilbenoids may have resveratrol monomeric biogenetic precursors. This is the initial report of a resveratrol monomer from the plant family Dipterocarpaceae, although resveratrol dimers, trimers, and tetramers were reported previously [27,28].

Although in our screening program to date, relatively few alkaloids have been investigated, a new analog **18** of the potent cytotoxic agent, tubulosine (**19**) was isolated and structurally characterized from

the stems of *Pogonopus speciosus* (Jack). K. Schum. (Rubaceae), collected in Panama. (–)-Tubulosine (**19**), also present in the same sample, was extremely potent in our cell culture panel, with the best activity demonstrated against the Lu1 human lung cancer cell line ($ED_{50} < 0.001 \mu\text{g/mL}$) [29]. It has been known for some time that tubulosine is active *in vivo*, when tested in the L1210 murine leukemia test system [30]. It is of interest that the novel compound (–)-1',2',3',4'-tetrahydro-tubulosine (**18**) was less potent as a cytotoxic agent in the cell panel in which it was evaluated by about two orders of magnitude, when compared with tubulosine (**19**). Compound **18** could prove useful as a negative control in future biological experiments utilizing the potent cytotoxin tubulosine (**19**) [29].

While all other plants mentioned in the present section of the chapter were obtained in tropical rainforest areas, separate samples of the flowers and leaves of *Ratibida columnifera* (Nutt.) Wood & Standl. (Compositae) were collected in Texas. Work-up of these samples resulted in the isolation and characterization of two new xanthanolides (**21** and **22**) and a new nerolidol sesquiterpene (**25**). In addition, three xanthanolides of previously known structure (**20**, **23** and **24**) were isolated and identified [31]. Xanthanolides **20–24** demonstrated broad cytotoxic activity when evaluated against a panel of human cancer cell lines. The nerolidol derivative **25** showed only weak general cytotoxicity. Of the cytotoxic isolates, the known compound **23** [9 α -hydroxy-*seco*-ratiferolide 5 α -O-(2-methylbutyrate)] was selected for further biological evaluation. In a 25 cell-line tumor panel, representing a diverse group of mouse and human tumors, fibroblasts, and normal bovine endothelial cells, compound **23** was found to exhibit a mean IC_{50} value of 1.46 μM , and exhibited a novel selectivity pattern, when effects on ovarian cancer cells (p53 mutant A2780R, parental wild-type A2780S), colon cancer cells (MDR⁺ HCT116/VVM46, MDR⁻ HCT 116), leukemic cells (HL-60, CCRF-CEM), and normal bovine aortic endothelial cells were examined [31].

It was then decided to examine the biological properties of 9 α -hydroxy-*seco*-ratiferolide 5 α -O-(2-methylbutyrate) (**23**) in greater detail. This sesquiterpene lactone was investigated for its effects on the cell cycle and on apoptosis, and was found to induce G₁ arrest at a concentration level of 1.16 $\mu\text{g/mL}$, in wild-type p53 A2780S cells. In p53 mutant A2780R cells, S traverse time was reduced, in addition to G₁ arrest. Both of these ovarian cancer lines underwent apoptosis when subjected to higher concentrations of **23**, with the p53 wild-type cells being more sensitive than the p53 mutant cells. In the concentration range 10–100 μM , compound **23** was found to have no effects on tubulin polymerization, on the inhibition of the catalytic ability of topoisomerase I and II enzymes, or on DNA intercalation. However, compound **23** was regarded as inactive when evaluated *in vivo* in two murine xenograft systems, namely, the M109 murine lung carcinoma and the HCT116 human colon carcinoma models [31].

Four bioactive flavonoids (**26–29**) were isolated from the combined leaves and stems of *Uvaria hamiltonii* Hook. f. et Th. (Annonaceae), collected in Thailand. The 5,7-dimethoxylated flavanones **26** and **27** were accorded the trivial names hamiltone A and B, respectively. Compounds **28** (hamiltrone), an aurone, and **29**, a chalcone analog of **28**, were also obtained in this investigation. All four compounds demonstrated strand-scission activity in the previously mentioned DNA strand-scission assay, with compound **28** being active at a dose of one-tenth of those of the other three compounds (activity of **26–29**: 1.1, 1.0, 10.0 and 0.6 'bleomycin units', respectively) [32].

A novel prenylated xanthone, tovobrevimastone (**30**), along with a known analog, manglexanthone (**31**), were isolated as cytotoxic constituents of the roots of *Tovomita brevistaminea* Engl. (Guttiferae), collected in Brazil. These compounds were evaluated as significantly cytotoxic ($EC_{50} < 5 \text{ mg/mL}$) for the KB (human oral epidermoid) cell line [33].

PROGRAM SUMMARY

It may be seen from the information provided in this brief review that tropical rainforest plants offer considerable chemical diversity in terms of the secondary metabolites that have been isolated and characterized recently as bioactive compounds, in our collaborative, multidisciplinary research program to search for novel antitumor agents of plant origin. Novel representatives have been described in each of the acylated oligorhamnoside, alkaloid (isoquinoline), cyclopenta[*b*]benzofuran, diterpenoid (*ent*-kaurene), flavonoid (aurone, chalcone, flavanone), oligostilbenoid, phenanthrenoid, sesquiterpene

(acyclic, xanthanolide), and xanthone structural classes. Several of these compounds have been selected for more advanced biological testing and mechanism of action studies.

ACKNOWLEDGEMENTS

The authors of this chapter wish to acknowledge grant U19-CA52956 from the National Institutes of Health, Bethesda, Maryland. Dr Qi Gao, Bristol-Myers Squibb, Pharmaceutical Research Institute, Wallingford, Connecticut is gratefully acknowledged for the X-ray crystallographic structure determination of domihinone. We are very grateful to our botanist collaborators in several countries for their cooperation in plant collection, and thank the many outstanding staff and faculty colleagues, postdoctoral associates, and graduate students who have participated in this collaborative research, and whose names are indicated in the bibliography below.

REFERENCES

- 1 S. H. Landis, T. Murray, S. Bolden, P. A. Wingo. *CA Cancer J. Clin.* **49**, 8–31 (1999).
- 2 D. M. Parkin, P. Pisani, J. Ferlay. *CA Cancer J. Clin.* **49**, 33–64 (1999).
- 3 M. E. Wall, M. C. Wani. *J. Ethnopharmacol.* **51**, 239–254 (1996).
- 4 J. M. Pezzuto. *Biochem. Pharmacol.* **53**, 121–133 (1997).
- 5 G. M. Cragg, D. J. Newman, R. B. Weiss. *Sem. Oncol.* **24**, 156–163 (1997).
- 6 Y.-Z. Shu. *J. Nat. Prod.* **61**, 1053–1071 (1998).
- 7 L. Long, A. D. Kinghorn. *Recent Res. Devel. Phytochem.* **2**, 35–71 (1998).
- 8 M. Suffness, G. M. Cragg, M. R. Grever, F. J. Grifo, G. Johnson, J. A. R. Mead, S. A. Schepartz, J. M. Venditti, M. Wolpert. *Int. J. Pharmacog.* **33**(Suppl.), 5–16 (1995).
- 9 G. A. Cordell, N. R. Farnsworth, C. W. W. Beecher, D. D. Soejarto, A. D. Kinghorn, J. M. Pezzuto, M. E. Wall, M. C. Wani, D. M. Brown, M. J. O'Neill, J. A. Lewis, R. M. Tait, T. J. R. Harris. In *Human Medicinal Agents from Plants* (A. D. Kinghorn, M. F. Balandrin, eds), Symposium Series no. 534, pp. 191–204. American Chemical Society, Washington, DC (1993).
- 10 A. D. Kinghorn, N. R. Farnsworth, C. W. W. Beecher, D. D. Soejarto, G. A. Cordell, J. M. Pezzuto, M. E. Wall, M. C. Wani, D. M. Brown, M. J. O'Neill, J. A. Lewis, J. M. Besterman. *Int. J. Pharmacog.* **33**(Suppl.), 48–58 (1995).
- 11 A. D. Kinghorn, N. R. Farnsworth, C. W. W. Beecher, D. D. Soejarto, G. A. Cordell, J. M. Pezzuto, M. E. Wall, M. C. Wani, D. M. Brown, M. J. O'Neill, J. A. Lewis, J. M. Besterman. In *New Trends in Natural Product Chemistry* (Atta-ur-Rahman, M. I. Choudhary, eds), pp. 79–94. Harwood Academic Publishers, Amsterdam (1998).
- 12 A. Artuso. *Drugs of Natural Origin. Economic and Policy Aspects of Discovery, Development, and Marketing*, pp. 3–4. The Pharmaceutical Products Press. Binghamton, New York (1997).
- 13 D. D. Soejarto. *J. Ethnopharmacol.* **51**, 1–5 (1996).
- 14 S. L. Bertha. *J. Ethnopharmacol.* **51**, 59–73 (1996).
- 15 M. E. Wall, M. C. Wani, D. M. Brown, F. Fullas, J. B. Oswald, F. F. Josephson, N. M. Thornton, J. M. Pezzuto, C. W. W. Beecher, N. R. Farnsworth, G. A. Cordell, A. D. Kinghorn. *Phytomedicine* **3**, 281–285 (1996).
- 16 G. A. Cordell, C. W. W. Beecher, A. D. Kinghorn, J. M. Pezzuto, H. L. Constant, H.-B. Chai, L. Fang, E.-K. Seo, L. Long, B. Cui, K. Slowing-Barillas. In *Studies in Natural Products Chemistry*, Vol. 19. *Structure and Chemistry (Part E)* (Atta-ur-Rahman, ed.), pp. 749–791. Elsevier Scientific Publishers, Amsterdam (1997).
- 17 G. A. Cordell, Y.-G. Shin. *Pure Appl. Chem.* **71**, 1089–1094 (1999).
- 18 H. L. Constant, C. W. W. Beecher. *Nat. Prod. Lett.* **6**, 193–196 (1995).
- 19 I.-S. Lee, L. A. Shamon, H.-B. Chai, T. E. Chagwedera, J. M. Besterman, N. R. Farnsworth, G. A. Cordell, J. M. Pezzuto, A. D. Kinghorn. *Chem.-Biol. Interact.* **99**, 193–204 (1996).
- 20 M. R. Boyd, K. D. Paull. *Drug Dev. Res.* **34**, 91–109 (1995).
- 21 M. G. Hollingshead, M. C. Alley, R. F. Camalier, B. J. Abbott, J. G. Mayo, L. Malspeis, M. R. Grever. *Life Sci.* **57**, 131–141 (1995).
- 22 B. Cui, H. Chai, T. Santisuk, V. Reutrakul, N. R. Farnsworth, G. A. Cordell, J. M. Pezzuto, A. D. Kinghorn. *Tetrahedron* **53**, 17625–17632 (1997).

- 23 S. K. Lee, B. Cui, R. R. Mehta, A. D. Kinghorn, J. M. Pezzuto. *Chem.-Biol. Interact.* **115**, 215–228 (1998).
- 24 L. Long, S. K. Lee, H.-B. Chai, P. Rasoanaivo, Q. Gao, H. Navarro, M. E. Wall, M. C. Wani, N. R. Farnsworth, G. A. Cordell, J. M. Pezzuto, A. D. Kinghorn. *Tetrahedron* **53**, 15663–15670 (1997).
- 25 H. Sugiyama, G. M. Ehrenfeld, J. B. Shipley, R. E. Kilkuskie, L.-H. Chang, S. M. Hecht. *J. Nat. Prod.* **48**, 869–877 (1985).
- 26 B. Cui, H. Chai, T. Santisuk, V. Reutrakul, N. R. Farnsworth, G. A. Cordell, J. M. Pezzuto, A. D. Kinghorn. *J. Nat. Prod.* **61**, 1535–1538 (1998).
- 27 E.-K. Seo, H. Chai, H. L. Constant, T. Santisuk, V. Reutrakul, C. W. W. Beecher, N. R. Farnsworth, G. A. Cordell, J. M. Pezzuto, A. D. Kinghorn. *J. Org. Chem.* **64**, 6976–6983 (1999).
- 28 E.-K. Seo, A. D. Kinghorn. *In Studies in Natural Products Chemistry*, Vol. 21. *Bioactive Natural Products* (Attatur-Rahman, eds). Elsevier Science Publishers, Amsterdam (in press).
- 29 A. Ito, Y.-H. Lee, H.-B. Chai, M. P. Gupta, N. R. Farnsworth, G. A. Cordell, J. M. Pezzuto, A. D. Kinghorn. *J. Nat. Prod.* **62**, 1346–1348 (1999).
- 30 W. R. Jondorf, B. J. Abbott, N. H. Greenberg, J. A. R. Mead. *Chemotherapy* **16**, 109–129 (1971).
- 31 B. Cui, Y.-H. Lee, H. Chai, J. C. Tucker, C. R. Fairchild, C. Raventos-Suarez, B. Long, K. E. Lane, A. T. Menendez, C. W. W. Beecher, G. A. Cordell, J. M. Pezzuto, A. D. Kinghorn. *J. Nat. Prod.* **62**, 1545–1550 (1999).
- 32 L. Huang, M. E. Wall, M. C. Wani, H. Navarro, T. Santisuk, V. Reutrakul, E.-K. Seo, N. R. Farnsworth, A. D. Kinghorn. *J. Nat. Prod.* **61**, 446–450 (1998).
- 33 E.-K. Seo, M. E. Wall, M. C. Wani, H. Navarro, R. Mukherjee, N. R. Farnsworth, A. D. Kinghorn. *Phytochemistry* **52**, 669–674 (1999).