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Recent studies on cytotoxic, anti-HIV and antimalarial agents from plants

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Abstract: Plants continue to provide key leading structures and therapeutic agents for a myriad of debilitating diseases. In this presentation, we will discuss the strategies being used, the structure elucidation and spectroscopic assignment of several classes of compounds with cytotoxic, anti-HIV and antimalarial activity, including xanthones and bisbenzylisoquinoline and Amaryllidaceae alkaloids, and we will provide some details of the bioassay systems being used. The application of various one- and two-dimensional nmr techniques to address opportunities for spectroscopic assignment will be described.

Since mankind first displayed susceptibility to disease and injury, the discovery of medicinal agents from renewable resources has been an essential and intrinsic aspect of all evolving cultures. Terrestrial plants are an especially viable opportunity for the discovery of biologically active natural products which may serve as commercially significant entities in their own right, or which may provide lead frameworks for the investigation of modified derivatives possessing enhanced activity and/or reduced toxicity. Three diseases of current concern on a global basis are cancer, AIDS and malaria, and there is a need for continued efforts to discover new template molecules from natural sources for each of these afflictions. This paper describes some of our recent strategies and efforts in each of these therapeutic areas.

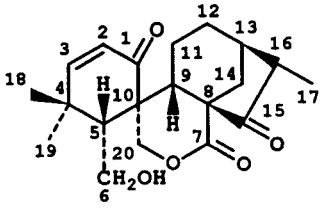
Anticancer Compounds

In the discovery of compounds active against the myriad of diseases known collectively as cancer, two plants, the periwinkle, *Catharanthus roseus*, and the May apple, *Podophyllum peltatum*, have provided important compounds (vincaleukoblastine and leurocristine in the former case and podophyllotoxin in the latter), which have stimulated research towards more active and less toxic derivatives. In the 1960s and 1970s, the search for anticancer agents continued through the use of one or two cytotoxicity assays coupled with an *in vivo* murine lymphocytic model. As cancer chemotherapy and cell biology evolved, it was found that a number of tumor types were resistant to chemotherapy and agents could be evaluated for their potential to intercede in discrete biological events in tumorigenesis or cell proliferation. Camptothecin was disclosed as an anticancer compound in 1966 and taxol in 1971 by Wall and Wani and their collaborators. But it was their unique mechanisms of action, namely, inhibition of topoisomerase I and of microtubule proliferation, respectively, which rekindled the interest in these compounds after early disappointments in clinical trials. Currently, these compounds and their derivatives are generating substantial chemical, biological, clinical, and even political interest (1).

Several years ago we established a battery of human cancer cell lines through which we could examine the possibility that certain agents might display selective cytotoxicity. While we have seen numerous instances of differential cytotoxicity, selective activity (i.e. 100-fold activity difference) has not been observed. We have however, adopted a cadre of human cancer cell lines (breast, lung, hormone-dependent breast, hormone-dependent prostate and colon) for routine testing of extracts and compounds. In addition, a number of mechanism-based assays, topoisomerase I, tubulin binding, protein kinase C inhibition, etc. have been established.

We have also examined some alternative strategies for discovering novel agents. One of these which has been successful is the HPLC-based DNA binding assay (2). In this technique, compounds in an extract which bind to DNA cause a diminution of "free" DNA which can be detected by HPLC as a percentage reduction in peak height. In this manner we isolated a group of macrocyclic polyamines, the budmunchiamines (3,4), and demonstrated that they possess a wide range of quite potent biological activities, including inhibition of platelet aggregation, human lymphcyte transformation, DNA polymerase, RNA polymerase and protein kinase C (5). This was a collaborative project between Professor Wagner's group in Munich and Dr. Neszmelyi's group in Budapest.

A number of traditional plants used against cancer for their active constituents have been evaluated using more classical *in vitro* assays. For example, plants in the genus *Isodon*, formerly *Rhabdosia*, were studied in collaboration with colleagues at the Kunming Institute of Botany. A number of new compounds were isolated displaying good cytotoxic activity, including laxiflorin C (1) from *I. eriocalyx* var. *laxiflora* (6). Although this is a well established group of diterpenes, our studies represent the first complete determination of their proton and carbon-13 nmr spectral properties, which was achieved through the use of one- and two-dimensional nmr techniques including ROESY, FLOCK and selective INEPT techniques, and a detailed analysis of coupling constants observed and calculated through the application of a molecular modeling program to define the minimum energy conformation.

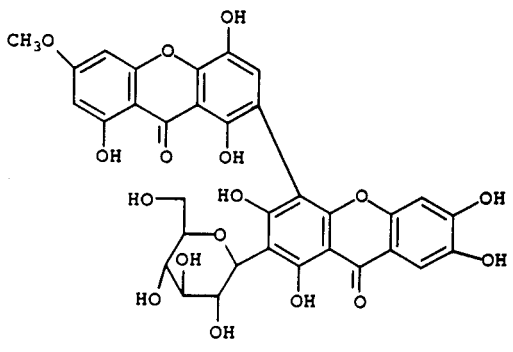


(1) Laxiflorin C

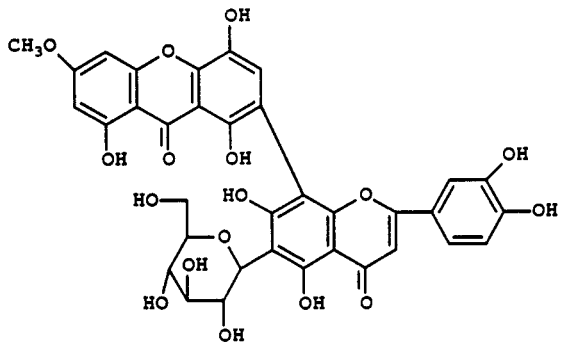
It was mentioned previously that many cancers in the clinical situation become resistant to chemotherapy. Another approach to drug discovery is to isolate compounds which can overcome this particular form of drug resistance. We have described thus far two such categories of compounds, the bisamides of *Aglaia* (7) and the lignans of *Phyllanthus* (8) using a KB cell line resistant to vincaleukoblastine.

HIV-Reverse Transcriptase Inhibition

AIDS is generally regarded as the most devastating global disease to have been identified in the past 30 years, and several compounds derived from natural sources are in various stages of investigation (9). As part of an effort to discover new natural product skeleta which might be of interest, we have established the HIV-reverse transcriptase assay (HIV-RT) in our laboratory (10), and more recently we have established additional RT assays, and are evaluating a wide range of extracts and isolates in these assays. We report here our results on the isolates of the genus *Swertia*, which is comprised of about 170 species of which 20 have been used in traditional Chinese medicine. In collaboration with Professor Yong-long Liu, we have isolated and characterized three novel compounds, swertiabisxanthone-1 from *S. macrosperma* (11), swertipunicoside (2) from *S. punicea* (12), and swertifrancheside (3) from *S. franchetiana* (13). These compounds represent new and novel skeleta; for example, swertifrancheside is the first flavone-xanthone C-glycoside.



(2) Swertipunicoside



(3) Swertifrancheside

As a part of our random testing program, we chose to examine the anti-HIV-reverse transcriptase activity of swertifrancheside (3) and swertipunicoside (2). Compound 3 showed an IC_{50} of 30.9 μg/ml, whereas swertipunicoside (2) was more active, showing an IC_{50} of 3.0 μg/ml. These are therefore some of the most active, plant-derived HIV-RT inhibitors yet isolated. These compounds provide a significant challenge in terms of both their structure elucidation from the aspects of placing the functional groups and determining the linkage sites on the two units. For the most part, these opportunities were addressed through the application of the selective INEPT technique, and their conformational peculiarities, including the doubling of certain portions of the carbon-13 nmr spectra, were studied through the application of molecular modeling. Studies to examine the viral mechanism of action of these compounds, as well as to isolate additional related compounds to explore the structure activity relationships, are underway.

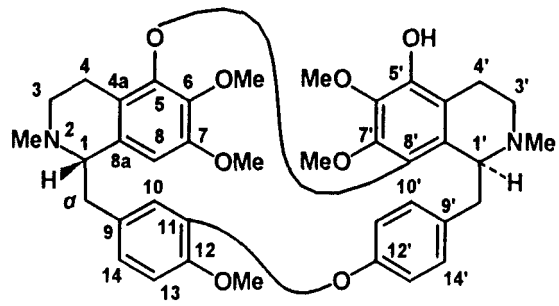
Antimalarial Compounds

Malaria afflicts up to 200 million people every year and several million die of malaria each year on a global basis. Resistance to chloroquine, and more recently quinine, has steadily increased. Yet programs for the discovery of new antimalarial lead structures from natural sources are extremely limited. This, in spite of the fact that two of the mainstay, clinically active antimalarial agents, quinine and artemisinin, were originally isolated from plants, and some other classes of compound, including the quassinoids and selected limonoids, have demonstrated activity against *Plasmodium falciparum* in culture (14). Recognizing this potential, we decided to initiate the investigation of some plants used in Thailand for the treatment of malaria or fevers. A group of sixteen plants was examined biologically for their cytotoxic (vs. KB) and antimalarial activities against chloroquine-resistant and sensitive clones of *P. falciparum*; of these, ten displayed activity. We chose not to investigate plants in the families Compositae, Meliaceae and Simaroubaceae because of their high level of cytotoxicity, selecting instead three plants in the family Menispermaceae, the tubers of *Stephania erecta* and *Stephania pierrei* and the roots of *Cyclea barbata*, and the bulbs of *Crinum amabile* in the Amaryllidaceae.

We also recognized that for a compound to be considered for future development, it would be necessary for it to be either more *in vitro* active than the existing agents, or less toxic, or, optimistically, both. All of the existing antimalarial agents exhibit some cytotoxicity, and we therefore developed a "Selectivity Index" (SI), a ratio of the ED_{50} in the KB cytotoxicity assay and the IC_{50} in the respective *Plasmodium falciparum* culture (both in ng/ml), in order to look for active compounds with reduced cytotoxicity.

From *S. erecta*, the bisbenzylisoquinoline alkaloids 2-noriso-tetrandrine (SI 95), 2-northalrugosine (SI 92), daphnandrine (SI 92), and 2-norobaberine (SI 130) were isolated as the principal active constituents together with 9 other alkaloids having SI values in the range 11 to 84 (14). Tetrandrine, for which a patent was issued (16), shows a SI of 12. *S. pierrei* yielded the aporphine alkaloids asimilobine (SI 21) and xylopine (SI 45)(17). *C. barbata* afforded limacine (SI 186), cycleapeltine (SI 76) and thalrugosine (SI 52)(18), and *C. amabile* afforded augustine (SI 4)(19).

The preliminary success of this work led us to examine the NMR parameters of the bisbenzylisoquinolines in order to completely assign, for the first time, all of the protons and carbons, and to investigate some unusual spectral features, such as the four missing aromatic protons in the spectrum of thalmirabine (4)(20). In collaboration with Professor Helene Guinaudeau we have also evaluated the range of antimalarial activity in the hope of developing some structure activity relationships. We therefore examined 37 alkaloids in 6 different structural classes (i.e. linkages). The most active compounds were malekulatine (SI 490), 2-norberbamine (SI 2,400), candicusine (SI 300), repandine (SI 350), thalmirabine (SI 220), 12-*O*-methyltricordatine (SI 200), and cycleanine (SI 460).



(4) Thalmirabine

In our studies thus far we have discovered four new groups of alkaloids which heretofore had not been known to possess antimalarial activity, namely, the 5,10b-ethanophenanthridines, aporphines, tetrahydroprotoberberines, and diverse bisbenzylisoquinolines.

Summary

Plants continue to be an excellent source of lead compounds against cancer, AIDS and malaria. Some new strategies which have led to the isolation of several highly active compounds have been described.

Acknowledgments

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REFERENCES

1. G.A. Cordell and M. Suffness. *Alkaloids (NY)* **25**, 1 (1985).
2. J.M. Pezzuto *et al. J. Nat. Prod.* **54**, 1522 (1991).
3. J.M. Pezzuto *et al. Heterocycles* **32**, 1961 (1991).
4. L.-Z. Lin *et al. Phytochemistry* **31**, 1795 (1992).
5. W. Mar *et al. J. Nat. Prod.* **54**, 1531 (1991).
6. H.-D. Sun *et al.* Unpublished results.
7. E. Saifah *et al. J. Nat. Prod.* **56**, 473 (1993).
8. A. Somanbandhu *et al. J. Nat. Prod.* **56**, 233 (1993).
9. R.F. Schinazi, *Nat. Prod. Antiviral Agents*. 1 (1992).
10. G.T. Tan *et al. J. Nat. Prod.* **54**, 143 (1991).
11. H.-M. Zhou *et al. Phytochemistry* **28**, 3569 (1989).
12. P. Tan *et al. J. Org. Chem.* **56**, 7130 (1991).
13. J.-N. Wang *et al. J. Nat. Prod.* In press.
14. D. Payne. *Parasitology Today* **2**, 241 (1987).
15. K. Likhitwitayawuid *et al. J. Nat. Prod.* **56**, 30 (1993).
16. K. Van Dyke, U.S. Patent 5,025,020; *Chem. Abstr.* **115**, 105992w (1991).
17. K. Likhitwitayawuid *et al. J. Nat. Prod.* **56**, 1468 (1993).
18. L.-Z. Lin *et al. J. Nat. Prod.* **56**, 22 (1993).
19. K. Likhitwitayawuid *et al. J. Nat. Prod.* **56**, 1331 (1993).
20. H. Guinaudeau *et al.* Unpublished results.
21. L.-Z. Lin *et al. Acta Chim. Sinica* **39**, 159 (1981).
22. L.-Z. Lin *et al.* Unpublished results.