A Multipronged Approach to the Study of Peruvian Ethnomedicinal Plants: A Legacy of the ICBG-Peru Project△

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A multidisciplinary and international team of scientists was assembled in the early 1990s to conduct an ethnobotanical study of plants used by the Aguaruna people of the Peruvian Amazon forest. The initial ethnobotanical project, carried out under the auspices of an International Cooperative Biodiversity Grant (ICBG), led to the collection of approximately 4000 plant species. Some members of the original team of scientists have continued this collaboration by focusing on potential sources of new anticancer, anti-infective, and wound-healing agents. This effort has uncovered several secondary metabolites representing a wide variety of chemical diversity. In this short review we describe some bioactive compounds of interest as part of our continuing collaboration.

Introduction

Nature is the ultimate source of chemical diversity. Synthetic libraries of organic compounds using combinatorial chemistry have yet to reach the ingenuity and complexity of the chemical diversity that is available through natural products produced by organisms after millions of years of evolution. The need to find and implement natural product sources of therapeutics should be expanded and accelerated in the face of the growing resistance of the current drugs of choice for many human diseases. Although conventional bioassay-guided isolation and structure identification of natural products is often criticized in terms of time, cost, and positive outcome,[1] this still remains the most effective methodology to find potential new drug candidates against different afflictions that continue to threaten the world's population, such as cancer and tropical diseases.[2]

Over a decade ago, a multidisciplinary and international group of scientists led by Walter H. Lewis (Washington University St. Louis), Abraham J. Vaisberg (Universidad Peruana Cayetano Heredia), and Gerardo Lamas (Universidad Nacional Mayor de San Marcos), under the auspices of an International Cooperative Biodiversity Groups (ICBG) project, embarked on an ethnobotanical study of Peruvian medicinal plants, guided by members of the Aguaruna community. The Aguaruna are one of the four tribes that constitute the Jívaro linguistic family, living in the upper Amazon basin and adjacent foothills of the eastern Andes Mountains of northern Peru and nearby Ecuador. Northern Peru, particularly the eastern slopes of the Andes Mountains and adjacent upper Amazon basin, is exceedingly rich in diverse woody plants. Only a small percentage (~2%) of these Peruvian species have been investigated chemically and/or biologically, and it is estimated that over 2000 plants in the Amazon region are used in traditional medicine.[3] A large number of the species chosen, prescreened for human use by the Aguaruna themselves, provided higher frequencies of bioactive secondary metabolites than those found in the flora as a whole.[4] Medicinal uses of plants by the Aguaruna are widespread, and their rich and varied plant pharmacopeia is still largely intact. The ethnobotanical focus of the ICBG-Peru project of the 1990s led to pioneering legal agreements with the Aguaruna community and the field collection of almost 4000 plant species. This international scientific network, forged as a result of the ICBG-Peru project, did not abate after the grant ended. On the contrary, the impressive collection of tropical plants acquired spurred an in-depth chemistry-based study, in which most of the original scientists, with the help of chemists and advisors, among which we proudly included Professor David G. I. Kingston, contributed to the isolation and identification of potential new anticancer, anti-infective, and wound-healing secondary metabolites. A common thread in our plant studies was the discovery of metabolites with an additional profile of bioactivities compared to the ones sought based on traditional use. In this communication, we would like to highlight some recent findings from our multidisciplinary and international group, a sequel of the ICBG-Peru project that has been invigorated by the contribution of new collaborators.

Recently Obtained Biologically Active Leads

During our search for cytotoxic agents from plants, we found that the ethanolic extract of the bark of *Uncaria guianesis* (Aubl.) Gmel (Rubiaceae), a plant used traditionally as an anti-inflammatory, contained the alkaloids uncarine C and uncarine E (**1**), possessing weak, yet selective, activity against the RS321 and RS322 yeast strains, with IC_{12} values of 380.3 and 325.9 μM for uncarines C and E, respectively. The uncarines also showed moderate cytotoxicity to several mammalian cancer cell lines, with IC_{50} values ranging from 46 to 114 μM (Table 1).[5] Another metabolite, the iridoid plumericin (**2**), previously isolated from the extract of the bark of *Himatanthus sucuuba* Spruce (M. Arg.) Woodson (Apocynaceae)[6] ("bellaco-caspi", a plant with a multitude of ethnomedicinal uses),[7] was responsible for the DNA-damaging

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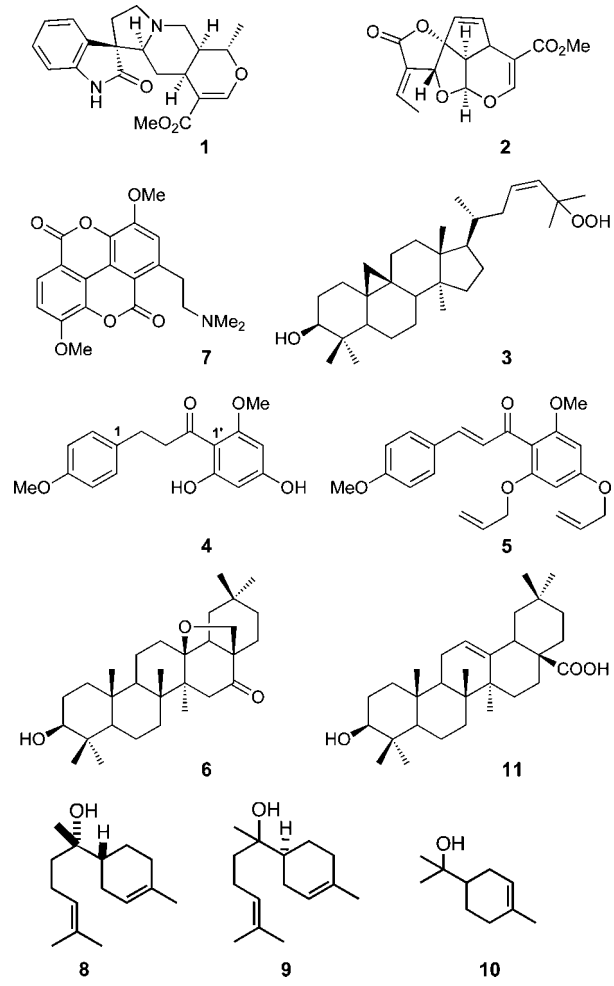
Table 1. Cytotoxicity of Compounds **1**−**5** and **8**.[a]

	GI_{50} (μM) in indicated cell line[b]									
compound	3T3	H460	ME180	DU145	MCF-7	M-14	HT-29	PC3	K562	Vero
1	62.4	46.1	67.9	114.0						
2	430.6	275.6	>430	>430	>430		>430			
3	82.6	67.4		78.7	>136	58.6	65.2	>136	65.2	>136
4	140.6	147.9		137.9	75.7	133.3	117.4	153.1	24.5	133.6
5	107.2	52.3		54.9	51.0	72.2	74.3	47.3	43.4	
8	54.7	32.8	18.2	25.8	30.0	15.1	28.9	28.5	40.5	

[a] The maximum concentration investigated was 136 μM. [b] Key to cell lines used: 3T3, BALB/3T3 clone A31 embryonic mouse fibroblast cells; H460, human large cell lung cancer; ME180, human cervical carcinoma; DU145, human prostate carcinoma; MCF-7, human breast adenocarcinoma; M-14, human melanoma; HT-29, human colon adenocarcinoma; PC3, human prostate adenocarcinoma; K562, human chronic myelogenous leukemia cells; Vero, normal African green monkey kidney epithelial cells.

activity in an engineered yeast that lacked the RAD52 DNA repair pathway. Plumericin (**2**) showed a relatively weak activity with an IC_{12} value of 241.2 μM for the RS321 yeast strain and GI_{50} values ranging from 275.6 to >430 μM against several mammalian cancer cell lines (Table 1).[8] The bark and stem of *Polylepis racemosa* R. & P. (Rosaceae) ("quenual"), a plant used by the local inhabitants to treat uterine cancer and inflammation, yielded pomolic acid as its main cytotoxic constituent.[9] Another triterpene acid, betulinic acid, isolated from the lianas of *Doliocarpus dentatus* (Aublet) Standley (Dilleniaceae) ("waúna"), used ethnobotanically to treat aftereffects of malaria, was found as its major cytotoxic principle.[10] When investigated against different types of mammalian cancer cell lines, pomolic acid showed cytotoxic values ranging from 17.6 to 52.9 μM, and betulinic acid from 23.6 to 136.9 μM.[9,10] The hydroperoxycycloartanes 24-hydroperoxycycloart-25-en-3β-ol and 25-hydroperoxycycloart-23-en-3β-ol (**3**), isolated from the extract of the whole plant of *Blepharodon nitidum* (Vell.) J. F. Macbr. (Asclepiadaceae), exhibited cytotoxic and anti-infective activities against axenic amastigotes of *L. amazonensis* and a multidrug-resistant (MDR) *Mycobacterium tuberculosis* (MTB) strain. The cytotoxic values shown by **3** ranged from 58.6 to >136.2 μM (Table 1) when screened against several cancer cell lines. Also, both compounds exhibited a relatively high leishmanicidal activity of 5.0 and 5.2 μM, respectively. However, while both of these hydroperoxides did nost show significant activity against a sensitive H_{37}Rv MTB, compound **3** showed a moderate activity (27.3 μM) against MDR-MTB strains. These two compounds may be regarded as reactive oxygen species (ROS) lipid hydroperoxides (considering the lipophilic saturated cycloartane group) or as producers of ROS, such as superoxide anions, hydroxyl radicals, and alkoxy radicals.[11]

One trademark of our approach to the study of traditional medicinal plants is that we have strived, inasmuch as it was possible, to screen crude extracts and partially purified fractions against a panel of assays related to several diseases. This approach produced interesting results, as in the case of the cytotoxic dihydrochalcones 2′,4′-dihydroxy-6′-methoxy-3,4-methylenedioxydihydrochalcone and 2′,4′-dihydroxy-4,6′-dimethoxydihydrochalcone (**4**), isolated from *Iryanthera jueruensis* Warb (Myristicaceae) ("untush tsempu").[10] These compounds served as a template for the search of chalcone analogues with selective and significant in vitro anti-*Trypanosoma cruzi* activity.[12] At first, we synthesized the isolated dihydrochalcones to confirm their structure. However, as we became aware of the known antiprotozoal activity of chalcones, we decided to screen the in vitro trypanoside activity of all of our synthetic intermediates against *T. cruzi* (Tulahuen C4) transfected with β-galactosidase (Lac Z), a strain used for high-throughput screening for assessing multiple chemical agents for their anti-*T. cruzi* efficacy. To our delight, the synthetic intermediates containing two allyloxy moieties on ring A (**5**) exhibited high trypanoside activity ranging from 1.5 to 20.3 μM, with selectivity indexes of up to 15.6 against normal African green monkey kidney epithelial cells (Vero). These chalcones, containing two allyloxy moieties on ring A and different electron-donor and/or electron-deficient substituents on ring B, yielded potentially useful drug candidates against Chagas' disease, and



because they can be made readily available through synthesis they could be appealing as potential therapeutic agents in Third World countries.[13]

The oleanane-type triterpene aegicerin (**6**), isolated from *Clavija procera* B. Stahl (Theophrastaceae), a plant used by the Aguaruna people to heal cutaneous leishmaniasis, exhibited a high level of antituberculosis activity in a tetrazolium microplate assay against 37 different sensitive and resistant *M. tuberculosis* strains, with activity values ranging between 3.5 and 6.8 μM.[14] Aegicerin had been isolated and identified over 40 years ago during a phytochemical investigation, but the anti-TB activity was unknown until our work was performed. This result resonates with Cordell and Colvard's observation that of all ethnomedical reports on about 14 300 species of plants in the NAPRALERT database, there has been no compound isolated and no biological work conducted for 8387 (58.6%) of them.[15]

A mainstay of our work with Peruvian medicinal plants has been the search for metabolites with wound-healing activity. Impaired

wound healing may cause severe health-related complications, such as infections and tissue necrosis. Because there are several stages in the cicatrization process—each of which is not fully understood—the use of animal models is necessary for full scientific assessment.[16] In vivo assays require a large amount of resources and materials. Thus, it should come as no surprise that the search for wound-healing agents is not only cost and labor intensive but also a slow, laborious process. Indeed, the vast majority of published papers using in vivo models have focused only on crude or partially purified plant extracts.[17] Although the merits of in vivo vs in vitro assays in wound-healing plants have been recently questioned, there are no commercial applications that we are aware of that use active principles found through in vitro assays. However, taspine (**7**), an alkaloid we isolated from the tree sap from *Croton lechleri* Müll. Arg. (Euphorbiaceae) ("sangre de grado"), through an in vivo-guided fractionation,[18] has been described in two patented wound-healing applications.[19] The emphasis of our international and multidisciplinary group has been to seek, insofar as it is possible, wound-healing compounds for which the structures are either commercially or readily available or easily derivatized. This line of thought was exemplified in our discovery of the terpenoid (+)-epi-α-bisabolol (**8**), the wound-healing active component in *Peperomia galioides* HBK (Piperaceae) ("congona"). This plant is used in Peruvian traditional medicine as a compress made from the crushed plant (except the roots) that is applied on cuts and wounds to accelerate healing; its juice is swallowed to treat gastric ulcers. We identified **8** as being responsible for the wound-healing activity in mice (ED_{50} 155 mg kg^{-1} mice). Finding this terpenoid as the active principle led to the screening of other, simpler terpenes with similar structural features to anymol. This effort led to the discovery of two new wound-healing agents, namely, the commercially available α-terpineol (**9**) and α-bisabolol (**10**).[20] The importance of our finding is that whereas **9** and **10** are inexpensive, **8** is not commercially available. Another case in point was the study of *Anredera diffusa* (Moq.) Sperling (Bassellaceae) ("lloto"), a plant for which the ethanolic extract exhibited significant cicatrizant activity in mice and was nontoxic in acute toxicity screening.[21] On the basis of our preliminary studies, we hypothesized that the wound-healing agent(s) in *A. diffusa* included saponin glycosides. We speculated that perhaps the aglycon(s) could also possess cicatrizant activity and decided to hydrolyze the ethanolic extract from the fresh leaves of *A. diffusa* prior to conducting an in vivo-guided fractionation, with the aim of simplifying the isolation procedure. This protocol led to the discovery of a new wound-healing agent, the inexpensive triterpenoid oleanoic acid (**11**).[22]

Conclusion

In summary, the ethnomedicinal search for biologically active metabolites from Peruvian plants, conducted in a multidisciplinary and international collaborative fashion, has uncovered principles for which no biological activity had been previously reported. Considering the small portion of Peruvian Amazonian plants studied relative to the large inventory collected by our group, it may be suggested that research on natural products sources of therapeutics has still a lengthy and prominent future.

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