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A Multipronged Approach to the Study of Peruvian Ethnomedicinal Plants: A Legacy of the ICBG-Peru Project^{\$}

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Abstract

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 want to describe some bioactive compounds of interest as part of our continuing collaboration.

Graphical abstract

^{\$}Dedicated to Dr. David G. I. Kingston of Virginia Polytechnic Institute and State University for his pioneering work in bioactive natural products.

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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 a 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 has 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, pre-screened 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 4,000 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 guianensis* (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 IC₁₂ values of 140 and 120 µg/mL for uncarines C and E, respectively. The uncarines also showed moderate cytotoxicity to several mammalian cancer cell lines, with IC₅₀ values ranging from 17 to 51 µg/mL (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 activity in an engineered yeast that lacked the RAD52 DNA repair pathway. Plumericin (**2**) showed a relatively weak activity with an IC₁₂ value of 70 µg/mL for the RS321 yeast strain and GI₅₀ values ranging from 80 to >125 µg/mL against several mammalian cancer cell lines (Table 1).⁸ The bark and stem of *Polylepis racemosa* R. & P. (Rosaceae), (“quenuál”), 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 after effects 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 ranged from 8.3 to 25 µg/mL, and betulinic acid from 10.8 to 62.5 µg/mL.^{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 26.9 to >62.5 µg/mL (Table 1), when screened against several cancer cell lines. Also, both compounds exhibited a relatively high leishmanicidal activity of 2.3 and 2.4 µg/mL, respectively. However, while both of these hydroperoxides did not show significant activity against a sensitive H₃₇Rv MTB, compound **3** showed a moderate activity (12.5 µg/mL) 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, in as much 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 template for the search of chalcone analogs 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 *Trypanosoma 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 μ M, 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 1.6 and 3.12 μ g/mL.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 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* H.B.K. (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 anamol. 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) ("loto"), a plant for which the ethanolic extract exhibited significant cicatrizant activity in mice, and was non-toxic in acute toxicity screening.²¹ Based on 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, oleanolic acid (**11**).²²

Conclusion

In summary, the ethnomedicinal search for biological 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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References and Notes

- (a) Bradshaw J, Butina D, Dunn AJ, Green RH, Hajek M, Jones MM, Lindon JC, Sidebottom PJ. *J Nat Prod.* 2001; 64:1541–1544. [PubMed: 11754607] (b) Koehn FE, Carter GT. *Nat Rev Drug Discov.* 2005; 4:206–220. [PubMed: 15729362]
- Kinghorn AD, Farnsworth NR, Soejarto DD, Cordell GA, Pezzuto JM, Udeani GO, Wani MC, Wall ME, Navarro HA, Kramer RA, Menendez AT, Fairchild CR, Lane KE, Forenza S, Vyas DM, Lam KS, Shu Y-Z. *Pure Appl Chem.* 1999; 71:1611–1618.(b) Paterson I, Anderson EA. *Science.* 2005; 310:451–453. [PubMed: 16239465] (c) Balunas MJ, Kinghorn AD. *Life Sci.* 2005; 78:431–441. [PubMed: 16198377] (d) Newman DJ, Cragg GM. *J Nat Prod.* 2007; 70:461–477. [PubMed: 17309302]
- Gentry AH. *Proc Natl Acad Sci USA.* 1988; 85:156–159. [PubMed: 16578826]
- (a) Gill, RC. *White Water Black Magic.* Victor Gollanez; London: 1941. p. 383(b) Lewis WH, Elvin-Lewis M, Gnerre MC, Fast DW. *Symp Bot Uppsala.* 1988; 28:189–196.(c) Lewis WH, Lamas G, Vaisberg A, Corley DG, Sarasara C, Albán J, Apanú R, Castro R, Crandall C, Elvin-Lewis M, Fritz W, Hammond G, Klueh P, Millán B, Okunade A, Pacheco V, Tovar O, Yarupaitán G. *Pharm Biol.* 1999; 37:69–83.
- Lee K, Zhou BN, Kingston DG, Vaisberg AJ, Hammond GB. *Planta Med.* 1999; 65:759–760. [PubMed: 10630124]
- Martin GE, Sanduja R, Alam M. *J Org Chem.* 1985; 50:2383–2386.
- Hammond GB, Vaisberg AJ, Fernandez ID, Maldonado H, Villegas LF, Zavaleta A. *J Ethnopharmacol.* 1997; 55:193–200. [PubMed: 9080340]
- Wood CA, Lee K, Vaisberg AJ, Kingston DGI, Neto CC. *Chem Pharm Bull.* 2001; 49:1477–1478. [PubMed: 11724243]
- Neto C, Vaisberg AJ, Zhou BN, Kingston DGI, Hammond GB. *Planta Med.* 2000; 66:483–484. [PubMed: 10909276]
- Aponte JC, Vaisberg AJ, Rojas R, Caviedes L, Lewis WL, Lamas G, Sarasara C, Gilman RH, Hammond GB. *J Nat Prod.* 2008; 71:102–105. [PubMed: 18163590]
- Aponte JC, Estevez Y, Gilman RH, Lewis WH, Rojas R, Sauvain M, Vaisberg AJ, Hammond GB. *Planta Med.* 2008; 74:407–410. [PubMed: 18484532]
- Aponte JC, Verástegui M, Málaga E, Zimic M, Quiliano M, Vaisberg AJ, Gilman RH, Hammond GB. *J Med Chem.* 2008; 51:6230–6234. [PubMed: 18798609]
- Hammond, GB., Aponte, JC., Gilman, RH., Sauvain, M. US Patent. 61,089,231. 2008.

14. Rojas R, Caviedes L, Aponte JC, Vaisberg AJ, Lewis WH, Lamas G, Sarasara C, Gilman RH, Hammond GB. *J Nat Prod.* 2006; 69:845–846. [PubMed: 16724857]
15. Cordell GA, Colvard MD. *J Ethnopharmacol.* 2005; 100:5–14. [PubMed: 16009517]
16. (a) Antoniadis HN. *Diabetes Rev.* 1995; 3:129–138.(b) Sen CK, Khanna S, Gordillo G, Bagchi D, Bagchi M, Roy S. *Alcohol Wine Health Dis.* 2002; 957:239–249.
17. Houghton PJ, Hylands PJ, Mensah AY, Hensel A, Deters AM. *J Ethnopharmacol.* 2005; 100:100–107. [PubMed: 16040217]
18. Vaisberg AJ, Milla M, Planas MC, Cordova JL, de Agusti ER, Ferreyra R, Mustiga MC, Carlin L, Hammond GB. *Planta Med.* 1989; 55:140–143. [PubMed: 2748730]
19. (a) Winter, REK., Kolodziej, SA., Lewis, WH. US Patent. 5474782. 1995. (b) Winter, REK., Okunade, AL., Elvin-Lewis, MPF., Lewis, WH., Kasiri, A. US Patent. 5932617. 1999.
20. Villegas LF, Marçalo A, Martin J, Fernández I, Maldonado H, Vaisberg AJ, Hammond GB. *J Nat Prod.* 2001; 64:1357–1359. [PubMed: 11678668]
21. Villegas LF, Fernández ID, Maldonado H, Torres R, Zavaleta A, Vaisberg AJ, Hammond GB. *J Ethnopharmacol.* 1997; 55:193–200. [PubMed: 9080340]
22. Moura-Letts G, Villegas LF, Marçalo A, Vaisberg AJ, Hammond GB. *J Nat Prod.* 2006; 69:978–979. [PubMed: 16792424]

Table 1Cytotoxicity of Compounds **1–5** and **8**.^a

compound	GI ₅₀ (μM) in indicated cell line ^b									
	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	

^aThe maximum concentration investigated was 136 μM.^bKey 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.