

(Report 1)

JAPAN SOCIETY FOR THE PROMOTION OF SCIENCE (JSPS)
Report on JSPS BRIDGE Fellowship Activities
 by individual BRIDGE Fellows

1. Fellow's BRIDGE Fellowship ID											
BR 130202											
2. Affiliated JSPS Alumni Association											
Member of the French JSPS Alumni association since 2003, treasurer (2006-2012), vice president of the association since 2012											
3. Name in Full											
LACAILLE-DUBOIS			Marie-Aleth								
FAMILY			First				Middle				
4. Host Researcher											
Name in Full					Affiliation						
MIYAMOTO Tomofumi					Graduate School of Pharmaceutical Sciences, Kyushu University, Fukuoka, Japan						
5. Period of BRIDGE Fellowship tenure											
From	29		July		2013	To	15		August	2013	
	Day		Month	/	Year		Day	/	Month	/	Year

7. Please write on the attached form.

8. Please write on the attached form.

9. Please write on the attached form.

Date: September 20th, 2013

NAME (Print): **LACAILLE-DUBOIS Marie-Aleth**

Signature: _____

(Notes)

1. Please send this completed form to both your host researcher and your affiliated alumni association as soon as possible after finishing your tenure under the BRIDGE Fellowship program.
2. The names and affiliations of Fellows and hosts and their reports may be given public access.

7. Research network created, sustained and/or strengthened with Japanese researchers through your visit. (Please add lines if needed)

7-1) Research network created:

My host researcher **Ass. Professor Tomofumi Miyamoto** (Laboratory of Natural Products Chemistry, Department of Medicinal Sciences, Graduate school of Pharmaceutical Sciences, Kyushu University, Fukuoka) allowed me to manage new contacts and to have discussions with colleagues of my discipline in Pharmacognosy.

After giving a presentation of my career and the areas of my research topics together with a presentation of the JSPS/BRIDGE program to the **dean Professor Kazuhide Inoue** and to the staff of the Faculty of Pharmacy during a council meeting with all the professors, I met **Professor Satoshi Morimoto** and **Ass. Professor Hiroyuki Tanaka** (Laboratory of Pharmacognosy).

We have valuable discussions on several areas of their outstanding research such as the biosynthesis of cannabinoids, the development of a combined technique using a rapid one-step immunochromatographic assay and indirect competitive ELISA for the rapid detection of natural compounds isolated from plants and also the study of single-chain variable fragment antibody against some ginsenosides as an effective tool for the determination of ginsenosides in various ginsengs. It was very interesting to share new ideas on the complementary aspect of our research in Pharmacognosy.

Furthermore, during a round trip in Japan, I visited **Professor Yoshinori Asakawa**, President of the Phytochemical Society of Asia, Director of the Institute of Pharmacognosy, Faculty of Pharmaceutical Sciences, Tokushima Bunri University. Pr Asakawa is actively involved in the isolation, structure elucidation, biological activity, biosynthesis, and chemosystematics of liverwort constituents. He has authored and co-authored more than 650 original papers, books and reviews. Our relevant scientific exchanges underlined our great complementary research interest since some species produce an important number of secondary metabolites with new skeletal terpenoids and aromatic compounds of which bibenzyls and bis(bibenzyls) are characteristic chemical markers of liverworts. They showed various biological activity such as anti-microbial, antiinfluenza, cytotoxicity, DNA polymerase, α -glucosidase and tubulin polymerization inhibitory activity, just to mention a few. I also had a chance to discuss with the PhD, post-doctoral students and to share our common research interest on natural products chemistry and biology and to see the high well equipped laboratory and well documented botanical garden of the faculty with important collections of Japanese medicinal plants.

Is there a possibility of the above network yielding an application for a JSPS program?

If yes, please state the name of the program and researchers who may participate on both sides.

7-2) Research network sustained:

I visited **Professor em. Haruki Yamada** in Tokyo (Kitasato University and the Kitasato Institute; chairman of Drugs for Neglected Diseases *initiative*, DNDi). He guided me for a very informative and interesting visit of the famous museum of Oriental Medicine, showing the history of oriental traditional medicine and its impact on Japan, together with the special collections of the specimens of herb medicines. I greatly appreciated to see the oldest Chinese pharmaceutical work dating from around the 1st century and which is the basis for all subsequent works of Pharmaceuticals. With beautiful illustrations, it describes 365 crude drugs (mineral, vegetable or animal in origin) to foster health, life, and to treat illness. The qualities, provenance and efficacy of each drug is described. After seeing the accomplishments in the dissemination of medical knowledge during centuries, the traditional medicine (Kampo) movement began from the 1930s to have a stable institutional existence, which is currently used in the hospital of traditional medicine.

Therefore, after the visit of this museum, Pr. Yamada showed me the Kampo Pharmacy. Here, I have seen an impressive number of drugs and the processing of some preparations ordered by practitioners. It was of great interest to see how around 10 drugs/preparation were weighted, mixed by a machine and then conditioned in small packages to be delivered to the patient. I had the opportunity to discuss with Pharmacy students who were training in this Pharmacy.

Then, Pr. Yamada introduced me to **Pr. Hiroaki Kiyohara** (Kitasato University, Kitasato Institute for Life Sciences, Laboratory of Biochemical Pharmacology for Phytomedicine & Graduate School of Infection Control science, Oriental Medicine Research Center. A presentation of my research activities was followed by a very fruitful discussion with Pr. Kiyohara who was very interested in our researches on phytopharmacology of saponins from medicinal plants, leading to a possible collaboration between our laboratories.

Another round trip into East Kyushu brings me to the Nagasaki International University, Faculty of Pharmaceutical Science, Huis Ten Bosch, Sasebo, Nagasaki, where **Professor Yukihiko Shoyama** was welcoming me. After visiting his laboratory, I delivered a lecture of one hour entitled "Phytopharmacological investigation of saponins from the African and Asian biodiversity: an update". Many researchers, PhD students and post doctoral students were very interested in this topic and asked a lot of questions. Pr. Shoyama showed me the wonderful botanical garden of the University which represents a great diversity of the Japanese flora.

I had also the chance to meet **Ass. Professor Osamu Morinaga**, Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, Nagasaki International University whose research area were very complementary of mines.

After visiting the colleagues in Sasebo, I met **Ass. Professor Koji Yamada**, Graduate school of biochemical Sciences, Nagasaki University in Nagasaki, who has in charge the responsibility of the Garden for Medicinal Plants. It was a great pleasure to visit this garden which possesses an impressive number of collections of Japanese plants and to discuss with Dr. Yamada on our common research areas in natural products chemistry.

Is there a possibility of the above network yielding an application for a JSPS program?

If yes, please state the name of the program and researchers who may participate on both sides.

7-3) Research network strengthened:

For over 20 years, my laboratory (Pharmacognosy, EA 4267 FDE/UFC, Faculty of Pharmacy, Burgundy University, Dijon) actively collaborates with the laboratory of **Ass. Professor Tomofumi Miyamoto** (Department of Natural Products, Graduate school of Pharmaceutical Sciences, Kyushu University, Fukuoka), since we share a common scientific interest in the chemistry and bioactivity of complex glycosides from medicinal plants and marine organisms. Our laboratories have acquired an expertise in this research field which is internationally recognized in more than 70 joint publications (2000-2013) in the best journals of the field (Phytochemistry, Journal of Natural Products, Helvetica Chimica Acta, Food Chemistry, etc....).

To reinforce our collaboration which started during my JSPS-post doctoral stay (one year in 1987) under the supervision of late Professor Tetsuya Komori followed by a three week stay in 2005 as invited Professor, we have explored several possibilities to find financial support for our common research project and grants for exchange or co-direction of PhD thesis. After this excellent collaborating partnership between our two groups having similar interests in Kyushu and in Burgundy, it is now time to do a review of all our results, and to extend this expertise to other researchers. Therefore, the main part of my stay during the JSPS/BRIDGE program was dedicated to strengthen this collaboration by the establishment of a work plan on a common innovative research program entitled "NMR Database construction for rapid structure determination of bioactive glycosides", which we intend to submit in a near future (France/Japan SAKURA collaborative project calls).

I also enlarged the research network with frequent exciting discussions and meetings with members of the laboratory, particularly **Assistant Professor Chiaki Tanaka** on the development of new technologies such as ESI-TOF-MS and 2D NMR experiments related to the structure elucidation of complexe molecules.

I have had also the opportunity to meet Japanese colleagues I have known for a long time, such as **Professor Riyuchi Higuchi**, who was my co-supervisor in the laboratory of late Professor Tetsuya Komori during my previous JSPS post-doctoral stay.

Is there a possibility of the above network yielding an application for a JSPS program?

yes

If yes, please state the name of the program and researchers who may participate on both sides.

SAKURA program

Associate Professor T. Miyamoto and Assistant Professor C. Tanaka from the Japanese side and two PhD students

Professor M.-A. Lacaille-Dubois and Professor A.-C. Mitaine-Offer from the french part and two PhD students

8. Results of your research and networking activities in Japan

During my stay in Japan under a JSPS-BRIDGE program, I visited five laboratories which were really of high level.

I delivered two lectures "Strategy for new drug discovery from natural products" (31/7/2013) in the department of Natural Products, Graduate school of Pharmaceutical Sciences, Kyushu University, Fukuoka and "Phytopharmacological investigation of saponins from the African and Asian biodiversity: an update" (2/8/2013) in Nagasaki International University, Faculty of Pharmacy (see abstract below), and one seminar in the Kitasato Institute, Kitasato University, Tokyo. These conferences in Japan with many attendees were made possible via the JSPS/BRIDGE program with which I was invited in Japan.

The main goal of my stay in Kyushu University was a discussion of our current collaborative research work with my research host, Ass. Prof. T. Miyamoto, and his co-workers who have an outstanding expertise and know-how in the structure elucidation of natural products by using complexed 1D and 2D NMR spectroscopic sequences and also mass spectrometry. The high quality of the 600 MHz 1D and 2D NMR spectra including COSY, TOCSY, NOESY, HSQC and HMBC and in mass spectra with FABMS, HRESIMS and ESI TOF MS, allowed the unambiguous determination of very complex structure of glycosides possessing from three to ten sugar units. Especially, the HSQC experiments gave the direct information of ^1H and ^{13}C chemical shifts in high sensitivity and rapidly, and the data are easily profiled using computer program such as Microsoft Excel.

By this way, we can define our future common project as "NMR Database construction for rapid structure determination of bioactive glycosides". In this project, we intend to construct the NMR database of oligosaccharide moieties of saponins and gangliosides, and to apply this database for rapid determination of novel glycosides obtained from various medicinal plants and echinoderms obtained in our laboratories.

We will put out this database on the Internet with the open access policy, and the researchers of glycosides can use and add the NMR data freely. It is necessary for us to develop an access-friendly database and easy-to-use data input-system. Furthermore, we must determine the unified standard for this database. For the reason stated above, our discussions about this database system in detail during my stay were fruitful.

In Japan, several groups have studied the structure determination of biologically active glycosides, and a large number of NMR data has been buried in the literature. In this program, we will use the NMR data not only ourselves but also data of other researchers. Until now, it has not been reported for the NMR database of glycosides, and if we are successful in the construction of this database, we will be a facility to offer structural information about glycoside regarding saponins and glycosphingolipids. In this context, the research network I have created, sustained and strengthened during my stay was in perfect agreement with our scientific program, we intend to submit as SAKURA project.

Abstract of my conference : "**Phytopharmacological investigation of saponins from the African and Asian biodiversity: an update**"

Pharmacognosy is probably the oldest pharmaceutical discipline. For thousands of years, man has used many plant extracts for medical treatments and nature is a huge reservoir of active molecules that are still being explored, for the search of new therapeutic strategies. Among these compounds, saponins known since antiquity for their detergent properties and toxicity to fish, have to date many uses in the food, therapeutic, and cosmetic areas.... Saponins, widely spread throughout the plant or marine organism kingdom are natural steroid- or triterpene-glycosides, having a large chemical diversity due to their structural lipophilic and hydrophilic parts. They are well known to interact with membranes of living cells resulting in membrane-disrupting properties with succeeding cell death (non-reversible effect), or transient change of membrane structure followed by specific biological effects. This class of natural phytochemicals presents a broad spectrum of biological properties including immunoadjuvant, immunomodulatory, antitumor, antiinflammatory, hypocholesterolemic, antiviral, antifungal, hypoglycemic properties, to mention just a few [1, 2].

We will present in this lecture the research strategy combining complementary disciplines, such as ethnobotany, phytochemistry and pharmacology, allowing isolation, characterization and biological assessment of saponins of interest. The extraction is carried out using conventional methods (maceration, soxhlet, refluxing) and more recently using the microwave technology. The isolation of such highly complex molecules containing up to eight or nine sugars has been greatly improved through the development of methods of preparative liquid chromatography, vacuum liquid chromatography, low and medium and high pressure liquid chromatography (VLC, MPLC, HPLC). The structural elucidation is performed by means of spectroscopic techniques such as 1D and 2D NMR (^1H , ^{13}C , DEPT, COSY, NOESY, TOCSY, HSQC, HMBC) and mass spectrometry (FAB-MS ESI) [3]. The biological tests include tests of hemolysis, cytotoxicity on human colon tumor cells, immunostimulatory assay on Jurkat cells, induction of apoptosis, and antifungal activity against pathogenic *Candida* yeasts. The discussion will also focus on the understanding of their mechanism of action and structure/activity relationships.

Examples will be chosen from plant saponins from the African and Asian biodiversity, which have been studied in our laboratory (Polygalaceae, Mimosaceae, Caryophyllaceae, Apiaceae, Dioscoreaceae, Liliaceae). Other saponin plants used in cosmetology (Rosaceae, Fabaceae, Araliaceae) will be also presented.

References: [1] Lacaille-Dubois, M.A. (2005) in: Atta-Ur-Rahman (Ed), Elsevier Science, Amsterdam, Vol. 32, pp 209-246. [2] Lacaille-Dubois, M.-A. (2013) In "Evidence and Rational Based Research on Chinese Drugs" Wagner H & Ulrich-Merzenich G (Eds), Springer-Verlag Wien, pp 87-135. [3] Mitaine-Offèr A.-C. *et al.* (2010) *Phytochemistry*, 71: 91-94.

9. Contributions to networking between researchers in your alumni association's country and colleagues in Japan

I hope that our emerging program will lead to some student exchanges in the future. In this frame, we discussed with my research host the possibilities of conventions between our university and Kyushu University in order to deliver PHD diploms in cotutelle. After coming back to my University, I will discuss on this topic with the President of our University who has in charge the international affairs in Burgundy university, and to discuss with him the possibilities of such bilateral conventions between Japan and France.

Concluding remarks

The most important during this JSPS/BRIDGE stay was to improve the academic network, that I established during my stays in Japan, one year in 1987 as a JSPS post-doc fellow and latter during three weeks in 2005 as invited Professor by the Kyushu University. With my research host, **Ass. Prof. T. Miyamoto**, we were able to define a common research project creating new developments on glycoside research which will be hopefully fruitful in a near future via the French Alumni association between French and Japanese Universities. I sincerely found this program as an excellent way to promote scientific and friendly exchanges between France and Japan. I'm very gratefull to all of them who took a large time in their busy schedules to take care of me and to have so many interesting discussions with me.

Again, I express my deepest thanks to JSPS for this exciting stay, both together from a scientific and cultural point of view, and hope that this program will be further developed for many JSPS fellows.