# Professional Advancement Virtual Engagement Series

CURE

## Seminar 9
## Establishing Your Research Group

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Assistant Professor, OB-GYN
University of California Los Angeles (UCLA)
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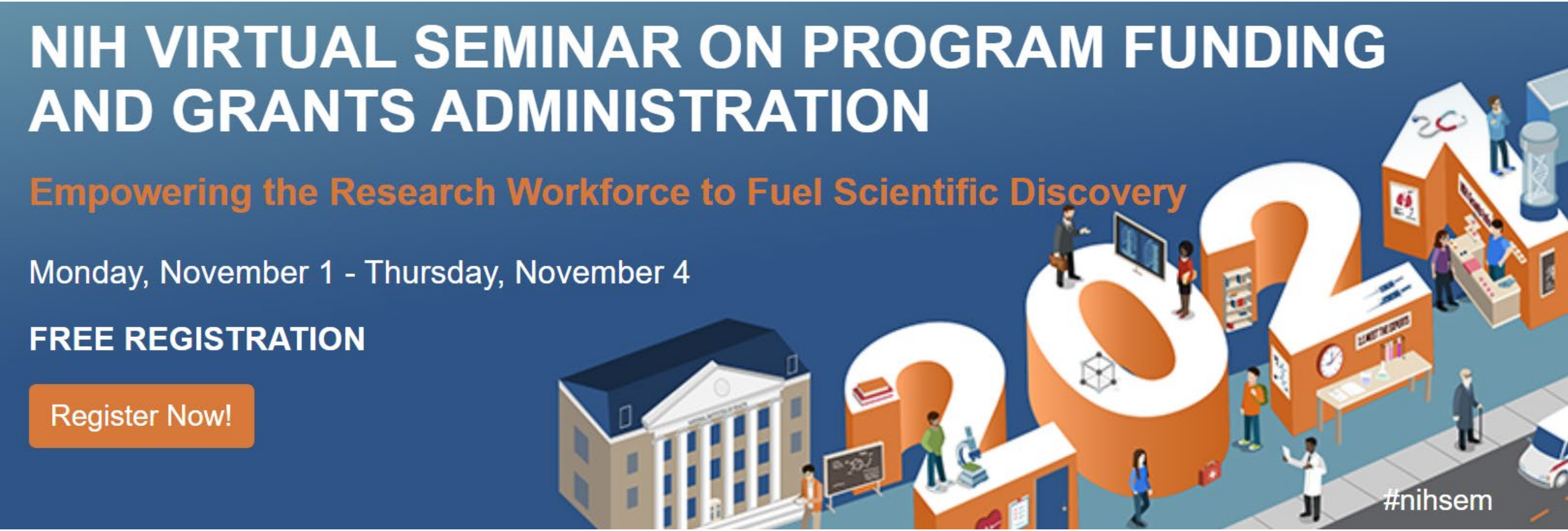
Associate Professor, College of Pharmacy
The Ohio State University

**October 25, 2021, 3 – 5 p.m. ET**

https://cbiit.webex.com/cbiit/j.php?MTID=m0bf6bf84698369e257dc7e4b811cf64d

NIH NATIONAL CANCER INSTITUTE

# Coming in November



## NIH VIRTUAL SEMINAR ON PROGRAM FUNDING AND GRANTS ADMINISTRATION

**Empowering the Research Workforce to Fuel Scientific Discovery**

Monday, November 1 - Thursday, November 4

**FREE REGISTRATION**

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https://nihvirtualseminar2021.vfairs.com/en/agenda-page
https://grants.nih.gov/2021-nih-virtual-seminar.htm#registration

# PAVES 9: Networking Session

## Ground Rules

Credit: COH

Credit: HCI

- Use respect to make this a safe space to chat
- Participate – listen and chat ☺
- Allow everyone the chance to speak
- Use of camera is highly encouraged

# PAVES 9: Networking Session

## Discussion Topic 1

Credit: COH

What was the most striking and/or useful take away message from today’s presentations for you?

Credit: HCI

How will you apply it to your own situation/career?

Professional
Advancement
Virtual
Engagement
Series

## Coming in November

Seminar 10

Cancer Systems Biology: Combining Computational Modeling, Experimental Methods, and Engineering to Address Cancer Biology Questions

**November 30, 2021, 3 – 5 p.m. ET**

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https://www.cancer.gov/about-nci/organization/crchd

**www.cancer.gov** **www.cancer.gov/espanol**

# Marta Epeldegui, PhD
UCLA AIDS Institute

# Research in the Epeldegui lab is focused on studying AIDS-malignancies

# How does HIV infection lead to B-cell lymphoma?

Many AIDS-associated cancers are associated with infection with oncogenic viruses (EBV, HHV8, HPV).

HIV infection results in the **loss of specific immunity to cells infected with such viruses**, leading to uncontrolled viral infection, transformation of infected cells, and cancer.

However, not all cancers seen in HIV+ populations are infected with an oncogenic virus.

HIV infection also results in the **chronic inflammation and immune system activation** (both antigen-specific and polyclonal), leading to DNA-modifying events and **oncogenic molecular errors** that result in cancer.

# AIDS-associated non-Hodgkin lymphoma

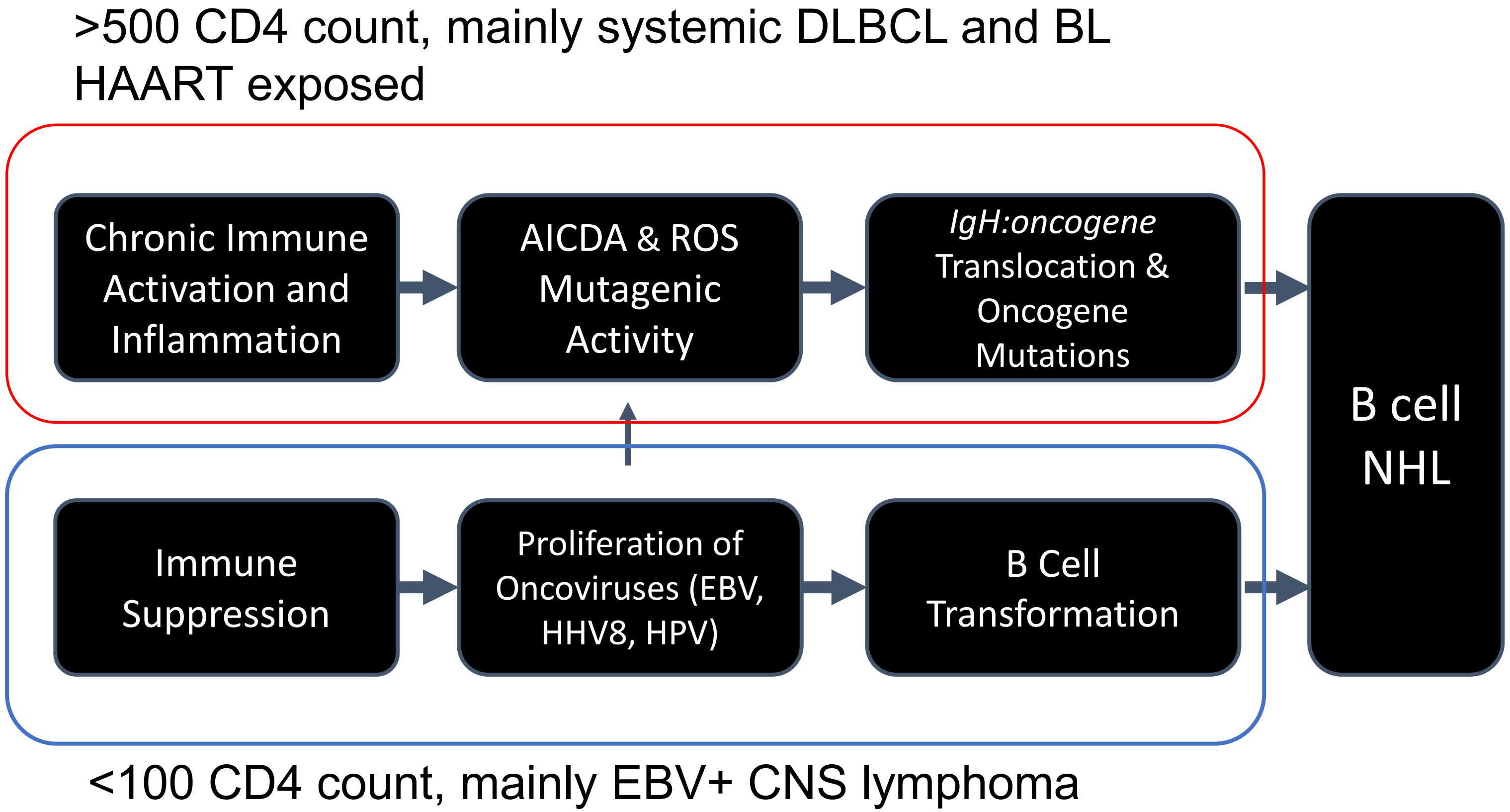
- Two major mechanisms are believed to contribute to the development of AIDS-NHL:

  1) loss of immunoregulatory control of EBV-infected B cells

  2) molecular lesions (oncogene mutations, *MYC:IgH* translocations) that result from errors in DNA modifying events (somatic hypermutation, class switch recombination) that occur during the process of B cell activation.

- Evidence that B cell activation is associated with AIDS-NHL comes from studies showing that:
  - serum levels of several cytokines (Breen, *et al*. CEBP 20:1303, 2011), and PBMC *AICDA* expression (Epeldegui, *et al*. AIDS 21:2265, 2007), are elevated prior to AIDS-NHL diagnosis
  - HIV can directly induce *AICDA* expression and production of cytokines, including IL10, in B cells (Epeldegui, *et al*. PLoS ONE 5:e11448, 2010).

# Model for Lymphomagenesis in HIV infection



>500 CD4 count, mainly systemic DLBCL and BL
HAART exposed

Chronic Immune Activation and Inflammation

AICDA & ROS Mutagenic Activity

*IgH:oncogene* Translocation & Oncogene Mutations

Immune Suppression

Proliferation of Oncoviruses (EBV, HHV8, HPV)

B Cell Transformation

B cell NHL

<100 CD4 count, mainly EBV+ CNS lymphoma

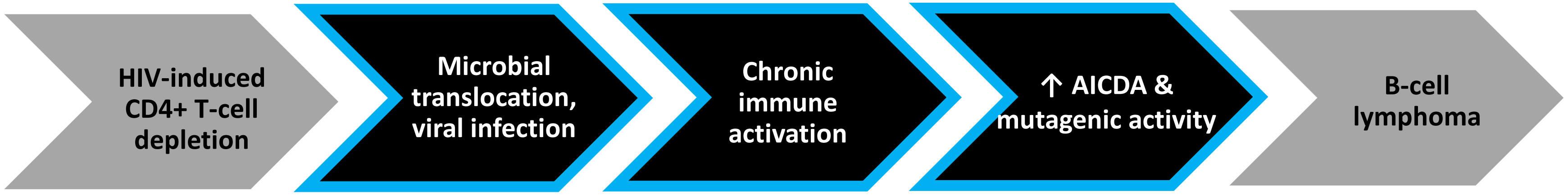
# Features of AIDS-NHL Subtypes

| **AIDS-NHL Subtype** | **Virus** | **%** | **CD4+ T cell count** | **Characteristic molecular lesions** | |
|---|---|---|---|---|---|
| | | | | **Oncogene hyper-mutation** | **Translocations** |
| Primary CNS lymphoma (25%-30%) | EBV | 100 | <50 | | |
| Primary effusion lymphoma (PEL) (rare) | EBV<br>KSHV | 50-80<br>100 | <50 | | |
| Systemic diffuse large B cell lymphoma (DLBCL) (25%-30%) | EBV | 40-60 | >200 | Majority | *BCL-6* mutations & translocation |
| Burkitt's lymphoma (25%-30%) | EBV | 30-50 | >200 | Some | *c-MYC:IgH* translocation |

Gopal, JCO, 2014; Chen, Oncolgist, 2007; Gaidano, Blood, 2003; Martinez, Opin Oncol, 2002

# Hypothesis:

## Elevated immune activation and inflammation (elevated serum cytokines and biomarkers) precede the diagnosis of AIDS-NHL


HIV-induced CD4+ T-cell depletion

Microbial translocation, viral infection

Chronic immune activation

↑ AICDA & mutagenic activity

B-cell lymphoma

- We and others have shown that:
  - ➢ AICDA is elevated prior to AIDS-NHL and that CD40L HIV virions induced AICDA expression in B cells (Epeldegui et al. AIDS, 2007, Epeldegui PLoS Ibe 2011, Imbeault et al. J. Virol. 2011)
  - ➢ Chronic Immune activation is elevated prior AIDS-NHL (Vedrame et al. CEBP 2013)
  - ➢ Microbial translocation is elevated prior to AIDS-NHL (Epeldegui et al. AIDS 2018, Marks et al. AIDS 2014). Microbial translocation is the translocation of commensal microbial products from the intestinal lumen into de systemic circulation in the absence of overt bacteremia.

# How do we study lymphomagenesis?

- By studying **biomarkers** as predictors of disease or as prognostic for treatment.
  - ✓**Immune biomarkers**, such as markers of inflammation , immune function (Th1, TH2, TFH, TH17), soluble receptors.
  - ✓Biomarkers **of microbial translocation**, Microbial translocation is present in different diseases: HCV, HBV and HIV infection, IBD, alcohol use, GVHD, Fatty liver disease.
  - ✓**Exosomes** as biomarkers, exosomes are extravesicles secreted by cells, that carry proteins in cell surface or as cargo, they can also carry DNA/RNA.
- By studying the role of regulatory B cells or Bregs, Bregs are characteristic for being $CD19^{+}CD38^{++}CD24^{++}$ and have regulatory function by secreting IL10 and expressing PDL1.Therefore having dual function in disease:
  1) Inhibiting T cell function through IL-10 and PDL1
  2) Activating B cells, since IL10 is a B cell stimulatory cytokine.

# A prospective study of serum microbial translocation biomarkers and risk of AIDS-related non-Hodgkin lymphoma

Marta Epeldegui[a], Larry Magpantay[a], Yu Guo[a], Gordana Halec[a], William G. Cumberland[a], Priscilla K. Yen[a], Bernard Macatangay[b], Joseph B. Margolick[c], Anne F. Rositch[c], Steven Wolinsky[d], Otoniel Martinez-Maza[a] and Shehnaz K. Hussain[e]

**Background:** Chronic immune activation is a harbinger of AIDS-associated non-Hodgkin lymphoma (AIDS-NHL), yet the underlying basis is unclear. Microbial translocation, the passage of microbial components from the gastrointestinal tract into the systemic circulation, is a source of systemic immune activation in HIV infection and may be an important contributor to chronic B-cell activation and subsequent AIDS-NHL development.

**Method:** We measured biomarkers of microbial translocation including bacterial receptors/antibodies, intestinal barrier proteins, and macrophage activation-associated cytokines/chemokines, in serum from 200 HIV-infected men from the Multicenter AIDS Cohort Study prior to their AIDS-NHL diagnosis (mean = 3.9 years; SD = 1.6 years) and 200 controls. Controls were HIV-infected men who did not develop AIDS-NHL, individually matched to cases on $CD4^+$ T-cell count, prior antiretroviral drug use, and recruitment year into the cohort.

**Results:** Biomarkers of bacterial translocation and intestinal permeability were significantly increased prior to AIDS-NHL. Lipopolysaccharide-binding protein (LPB), fatty acid-binding protein 2 (FABP2), and soluble CD14 were associated with 1.6-fold, 2.9-fold, and 3.7-fold increases in AIDS-NHL risk for each unit increase on the natural log scale, respectively. Haptoglobin had a 2.1-fold increase and endotoxin-core antibody a 2.0-fold decrease risk for AIDS-NHL (fourth versus first quartile). Biomarkers of macrophage activation were significantly increased prior to AIDS-NHL: B-cell activation factor (BAFF), IL18, monocyte chemoattractant protein-1 (MCP1), tumor necrosis factor-α (TNFα), and CCL17 had 2.2-fold, 2.0-fold, 1.6-fold, 2.8-fold, and 1.7-fold increases in risk for each unit increase on the natural log scale, respectively.

**Conclusion:** These data provide evidence for microbial translocation as a cause of the systemic immune activation in chronic HIV infection preceding AIDS-NHL development.

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*AIDS* 2018, **32**:945–954

CLINICAL CANCER RESEARCH | TRANSLATIONAL CANCER MECHANISMS AND THERAPY

# Immune Activation and Microbial Translocation as Prognostic Biomarkers for AIDS-Related Non-Hodgkin Lymphoma in the AMC-034 Study

Laura E. Martínez[1,2], Shelly Lensing[3], Di Chang[3], Larry I. Magpantay[1,2], Ronald Mitsuyasu[1], Richard F. Ambinder[4], Joseph A. Sparano[5], Otoniel Martínez-Maza[1,2,6], and Marta Epeldegui[1,2,6]

## ABSTRACT

**Purpose:** AIDS-related non-Hodgkin lymphoma (ARL) is the most common cancer in HIV-infected individuals in the United States and other countries in which HIV-positive persons have access to effective combination antiretroviral therapy (cART). Our prior work showed that pretreatment/postdiagnosis plasma levels of some cytokines, such as IL6, IL10, and CXCL13, have the potential to serve as indicators of clinical response to treatment and survival in ARL. The aims of this study were to identify novel prognostic biomarkers for response to treatment and/or survival in persons with ARL, including biomarkers of microbial translocation and inflammation.

**Experimental Design:** We quantified plasma levels of several biomarkers (sCD14, LBP, FABP2, EndoCab IgM, IL18, CCL2/MCP-1, sCD163, IP-10/CXCL10, TARC/CCL17, TNFα, BAFF/BLyS, sTNFRII, sCD44, and sIL2Rα/sCD25) by multiplexed immunometric assays (Luminex) or ELISA in plasma specimens obtained from ARL patients enrolled in the AMC-034 trial, which compared infusional combination chemotherapy (EPOCH: etoposide, vincristine, doxorubicin, cyclophosphamide, and prednisone) with concurrent or sequential rituximab. Plasma was collected prior to the initiation of therapy ($n = 57$) and after treatment initiation ($n = 55$).

**Results:** We found that several biomarkers decreased significantly after treatment, including TNFα, sCD25, LBP, and TARC (CCL17). Moreover, pretreatment plasma levels of BAFF, sCD14, sTNFRII, and CCL2/MCP-1 were univariately associated with overall survival, and pretreatment levels of BAFF, sTNFRII, and CCL2/MCP-1 were also associated with progression-free survival.

**Conclusions:** Our results suggest that patients with ARL who responded to therapy had lower pretreatment levels of inflammation and microbial translocation as compared with those who did not respond optimally.

SCIENTIFIC REPORTS

Corrected: Author Correction

OPEN

# Elevated numbers of PD-L1 expressing B cells are associated with the development of AIDS-NHL

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Marta Epeldegui[1,2,3], David V. Conti[4], Yu Guo[3], Wendy Cozen[4,5], Manuel L. Penichet[1,2,6,7,8] & Otoniel Martínez-Maza[1,2,3,7,9]

The risk for non-Hodgkin lymphoma (NHL) is markedly increased in persons living with human immunodeficiency virus (HIV) infection, and remains elevated in those on anti-retroviral therapy (cART). Both the loss of immunoregulation of Epstein-Barr virus (EBV) infected cells, as well as chronic B-cell activation, are believed to contribute to the genesis of AIDS-related NHL (AIDS-NHL). However, the mechanisms that lead to AIDS-NHL have not been completely defined. A subset of B cells that is characterized by the secretion of IL10, as well as the expression of the programmed cell death ligand-1 (PD-L1/CD274), was recently described. These PD-L1$^+$ B cells can exert regulatory function, including the dampening of T-cell activation, by interacting with the program cell death protein (PD1) on target cells. The role of PD-L1$^+$ B cells in the development of AIDS-NHL has not been explored. We assessed B cell PD-L1 expression on B cells preceding AIDS-NHL diagnosis in a nested case-control study of HIV+ subjects who went on to develop AIDS-NHL, as well as HIV+ subjects who did not, using multi-color flow cytometry. Archival frozen viable PBMC were obtained from the UCLA Multicenter AIDS Cohort Study (MACS). It was seen that the number of CD19$^+$CD24$^{++}$CD38$^{++}$and CD19$^+$PD-L1$^+$cells was significantly elevated in cases 1–4 years prior to AIDS-NHL diagnosis, compared to controls, raising the possibility that these cells may play a role in the etiology of AIDS-NHL. Interestingly, most PD-L1$^+$ expression on CD19$^+$ cells was seen on CD19$^+$CD24$^{++}$CD38$^{++}$ cells. In addition, we showed that HIV can directly induce PD-L1 expression on B cells through interaction of virion-associated CD40L with CD40 on B cells.

# How do we study lymphomagenesis?

- By studying follicular CD8 T cells ($CD8^+CXCR5^+BCL\text{-}6^+PD1^+$):
  - ✓fCD8 T cells are thought to be induced with inflammation and immune activation and are present in the B cell follicles.
  - ✓fCD8 T cells have the capability to interact with B cells and induce them to secrete antibodies, more like a helper T cell.
  - ✓fCD8 T cells may be important in diseases associated with B cell activation such as: autoimmune disease, HIV infection and cancer.

- By studying mouse models of lymphoma:
  - ✓Using the Humanize mouse model and immune deficient mice as models for lymphomagenesis.

**MOLECULAR CANCER THERAPEUTICS** | SMALL MOLECULE THERAPEUTICS

# Targeting TfR1 with the ch128.1/IgG1 Antibody Inhibits EBV-driven Lymphomagenesis in Immunosuppressed Mice Bearing EBV$^{+}$ Human Primary B-cells

Laura E. Martínez[1,2], Tracy R. Daniels-Wells[3], Yu Guo[1,2], Larry I. Magpantay[1,2], Pierre V. Candelaria[3], Manuel L. Penichet[2,3,4,5,6], Otoniel Martínez-Maza[1,2,4,5,7], and Marta Epeldegui[1,2,4]

## ABSTRACT

Epstein–Barr virus (EBV) is a human gammaherpesvirus associated with the development of hematopoietic cancers of B-lymphocyte origin, including AIDS-related non-Hodgkin lymphoma (AIDS-NHL). Primary infection of B-cells with EBV results in their polyclonal activation and immortalization. The transferrin receptor 1 (TfR1), also known as CD71, is important for iron uptake and regulation of cellular proliferation. TfR1 is highly expressed in proliferating cells, including activated lymphocytes and malignant cells. We developed a mouse/human chimeric antibody targeting TfR1 (ch128.1/IgG1) that has previously shown significant antitumor activity in immunosuppressed mouse models bearing human malignant B-cells, including multiple myeloma and AIDS-NHL cells. In this article, we examined the effect of targeting TfR1 to inhibit EBV-driven activation and growth of human B-cells *in vivo* using an immunodeficient NOD.Cg-*Prkdc*$^{scid}$ *Il2rg*$^{tm1Wjl}$/SzJ [NOD/SCID gamma (NSG)] mouse model. Mice were implanted with T-cell–depleted, human peripheral blood mononuclear cells (PBMCs), either without EBV (EBV$^{-}$), or exposed to EBV *in vitro* (EBV$^{+}$), intravenously via the tail vein. Mice implanted with EBV$^{+}$ cells and treated with an IgG1 control antibody (400 μg/mouse) developed lymphoma-like growths of human B-cell origin that were EBV$^{+}$, whereas mice implanted with EBV$^{+}$ cells and treated with ch128.1/IgG1 (400 μg/mouse) showed increased survival and significantly reduced inflammation and B-cell activation. These results indicate that ch128.1/IgG1 is effective at preventing the growth of EBV$^{+}$ human B-cell tumors *in vivo*, thus, indicating that there is significant potential for agents targeting TfR1 as therapeutic strategies to prevent the development of EBV-associated B-cell malignancies.

**Significance:** An anti-TfR1 antibody, ch128.1/IgG1, effectively inhibits the activation, growth, and immortalization of EBV$^{+}$ human B-cells *in vivo*, as well as the development of these cells into lymphoma-like tumors in immunodeficient mice.

# How it all started?

# Early career

- Undergraduate in Biology at the Universidad Complutense de Madrid.
- Master's degree in Developmental Biology at the Universidad Complutense de Madrid.
- PhD at University of California Los Angeles in Martinez-Maza lab.
    - ✓UCLA AIDS Institute Esther Hays Student Research Award.
    - ✓University AIDS Research Program (UARP) now the California HIV/AIDS Research Program (now CHRP) Fellowship.
    - ✓NIAID Clinical Immunology Training Grant (T32)
- Postdoc at the University of California Los Angeles Uittenbogaart lab.
    - ✓ NRSA Rheumatology Training Grant (T32)
    - ✓ NRSA Tumor Immunology Training Grant (T32)

# Career Development

- UCLA CFAR career development supplement Award.
- NCI CURE career development supplement Award
- R21- Exploratory Grant Award to Promote Workforce Diversity in Basic Cancer Research. R21-CA220475 (PI: Epeldegui)
- AIDS Malignancies Consortium (AMC) Lab Translational Fellowship.
- All this led to getting my first R01.

# Things that helped me succeed

- Mentors- they are key to your success. Good mentors are invested in your success for you not for them.

- NIH/NCI programs and opportunities- NIH/NCI has lots of opportunities. Program officers are a great resource.

- Perseverance- don’t let negative news get to you, dwell for a little then move on and always learn from your failures.

- Collaborators- they are also important on your success. Surround yourself with collaborators that complement your research. It is so much more fun to do science collaborating.

- Take a break- sometimes taking a break and doing something it makes you feel good may be the best medicine.

# Things that I am working on

- Patience- I observe that senior faculty have much more patience.
- It’s ok to say no- be realistic with what you can accomplish.
- Let go- it’s ok to sometimes not to have everything under control.
- Mentoring- pass on your knowledge onto others, try to change things that don’t work.
- Educate on Diversity- talk about it, why is it important, try to break the cycle.
- Be supportive- everyone is different.

# Working on establishing my research group.

Organization of the Epeldegui lab:

1) Biomarker arm:

- ✓We collaborate with groups from all the US measuring biomarkers (mainly epidemiologist).
- ✓Serve as the biomarker core for the AIDS Malignancies Consortium.
- ✓Work with cohorts (MACS, AMC,…) on developing new biomarkers.

2) Basic research:

- ✓ Studying pathogenesis and how AIDS-NHL develops.

# Working on establishing my research group.

How I am making it work:

✓I share resources with my mentor.

✓Have a lab manager that keeps all the lab maintenance and biomarker work moving.

✓My current postdoc was an undergraduate that worked with me when I was a graduate student.

✓Undergraduates are an important part of the lab (volunteers or work study)

✓Currently looking for another postdoc and a technician.

✓It’s hard to balance how much work needs to be done and how much you can afford

# Plans for the future….

Keep it up!

**Martinez-Maza/ Epeldegui Lab**
Laura Martinez
Larry Magpantay
Yu Guo
Ruchal Patel

**AMC**
Shelly Lensing
Di Chang
Joseph Sparano
Ron Mitsuyasu
Richard Ambinder
Jeff Bethony

**ACSR**
Paige Bracci
Jeff Bethony
Mike McGrath

**MACS**
Roger Detels
Otoniel Martinez-Maza
Shehnaz Hussain

**UCI**
Wendy Cozen

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NCI- AMC Lab/Translational Fellowship

NCI- UCLA JCCC P30CA016042-S

NIH-NCI, R21-CA220475
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NCI- R01CA228157-S02

UCLA AIDS INSTITUTE

UCLA Jonsson Comprehensive Cancer Center

THE OHIO STATE UNIVERSITY

# Making Pieces of the Challenging Puzzles of Natural Product Research

**Dr. Liva Harinantenaina Rakotondraibe**

**PAVES 9: October 25, 2021**

# Making Pieces of the Challenging Puzzles of Natural Product Research

PAVES 9

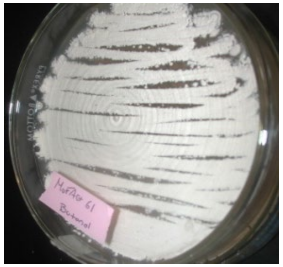
**Liva Harinantenaina Rakotondraibe**

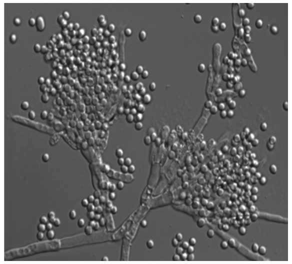
Associate Professor

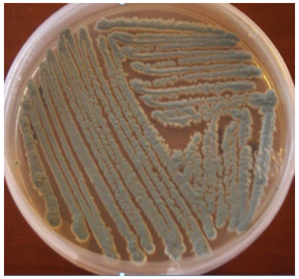
Division of Medicinal Chemistry and Pharmacognosy

# Natural Products

**Higher Plants**







**Liverworts**

**Marine organisms and their microbial associates**

**Bioactive compounds**

# Outline

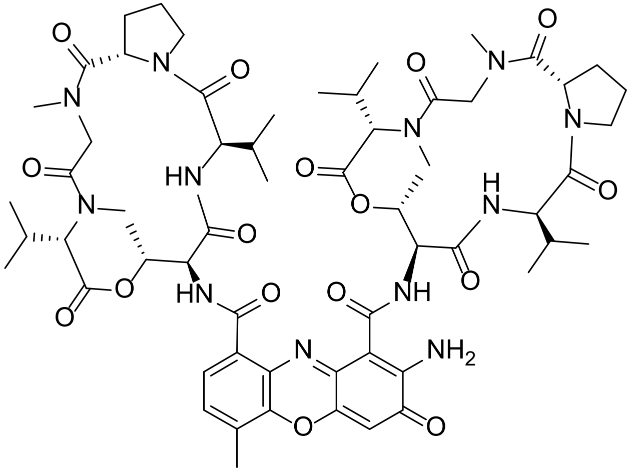
- **Natural products are prominent sources of bioactive compounds**

- **U.S. endemic lichens and their mycobionts are promising source of cytotoxic compounds**

- **Development of Total Correlation Spectroscopy (TOCSY), a Nuclear Magnetic Resonance (NMR)-based dereplication technique to speed up the discovery of new and bioactive compounds**

# Why Natural Products?

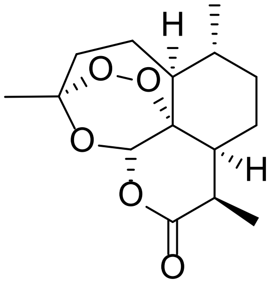
## They are sources of many FDA approved drugs



**Actinomycin D**

**Polypeptide antitumor antibiotic**

***Streptomyces* sp.**

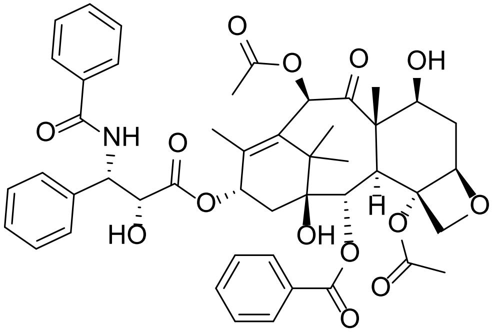


**Artemisinin**

**Antimalaria**

***Artemisia annua***

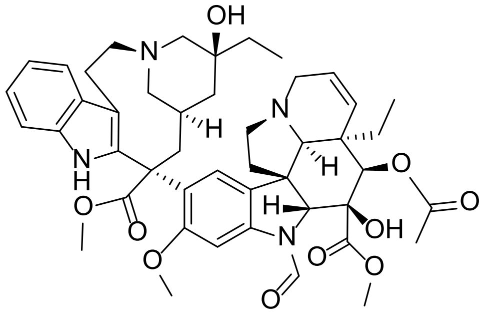
**Nobel winner 2015**



**Paclitaxel**

**(Taxol)**

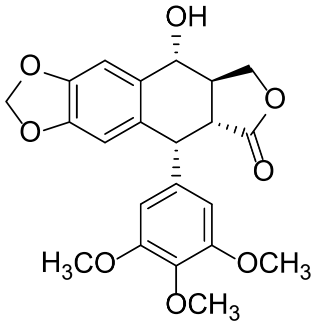
***Taxus brevifolia***



**Vincristine**

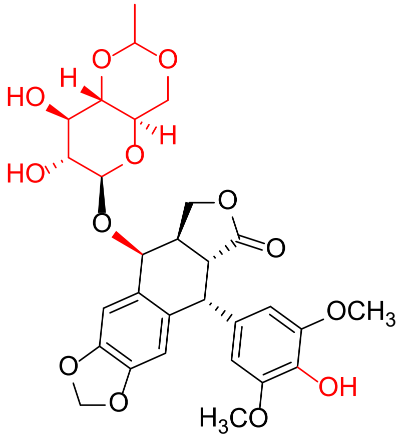
***Catharanthus roseus***

**Madagascar periwinkle**



**Podophyllotoxin**

***Podophyllum* sp.**



**Etoposide**

**Epipodophyllotoxin derivative**

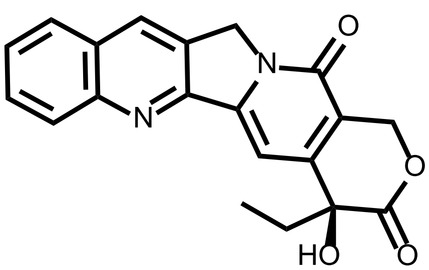
Newmann, D.J and Cragg, G.M. *J. Nat. Prod.* 79, 629-661 (2016)

# Why Natural Products?

## They are sources of many FDA approved drugs

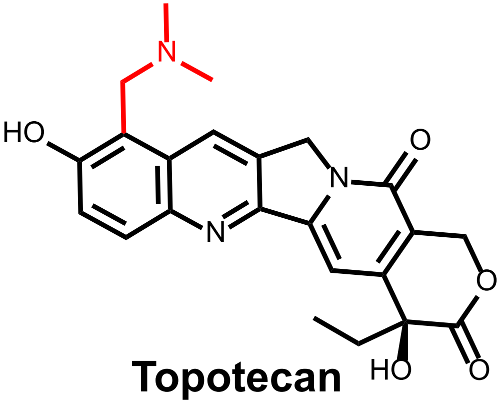
**Derivatization at one site of bioactive natural product affords more active/selective compound(s)**

**Solubility, stability and side effect issues → Development of derivatives: Topotecan, Irinotecan**



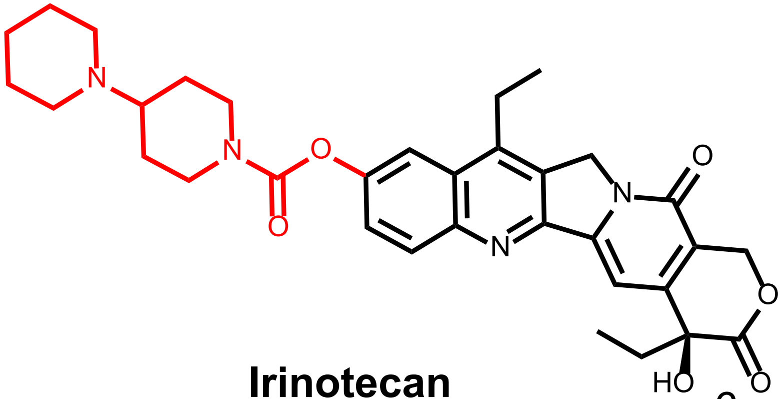
**Camptothecin**

*Camptotheca acuminate* Decne. (Nyssaceae)



**Topotecan**

**Ovarian, cervical and small-cell lung cancers**



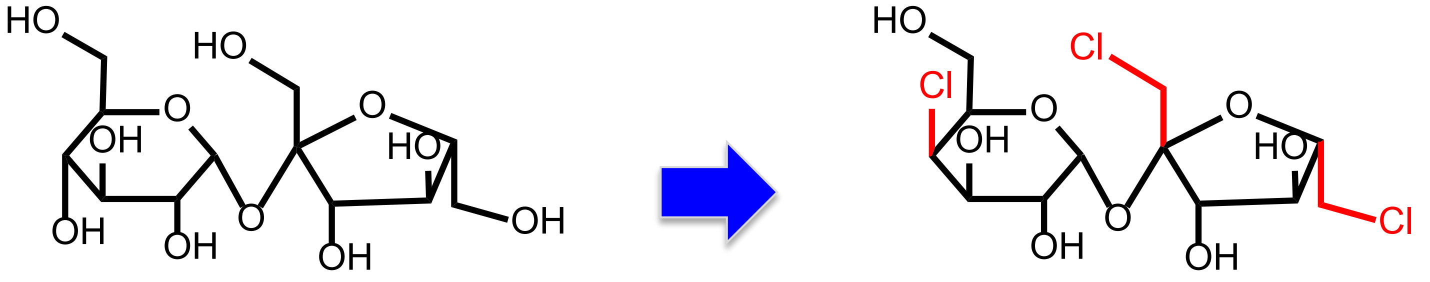
**Irinotecan**

**Metastatic colorectal cancer**

# Why Natural Products?

## They are sources of many FDA approved drugs

## No calorie Sweetener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**Sucrose**

**Sucralose**

*Saccharum officinarum* (Poaceae)

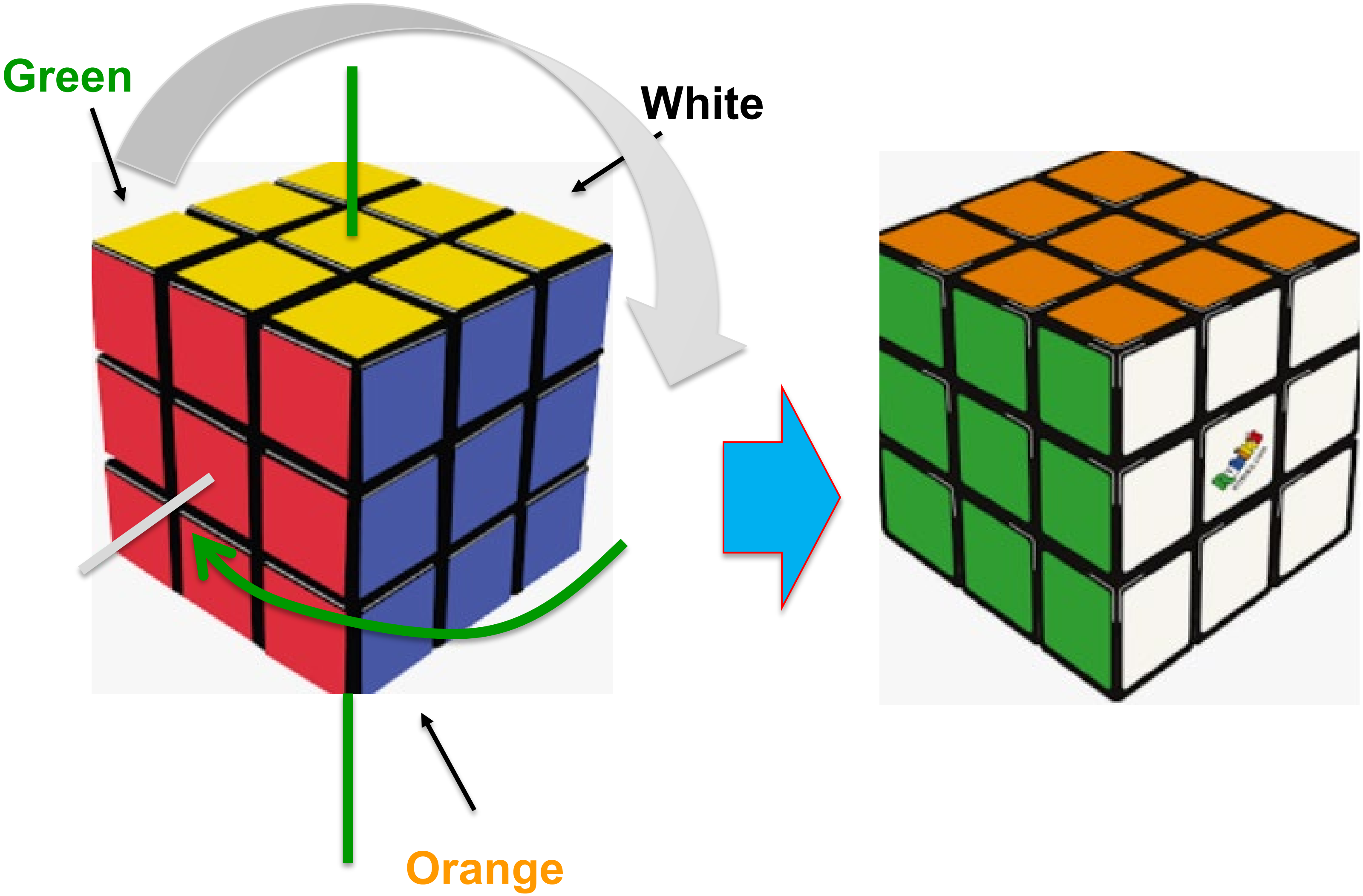
Splenda
No Calorie Sweetener
Splenda
No Calorie Sweetener

- Natural products produce bioactive compounds that can be used as medicines, foods, and insecticides or insect repellent,…

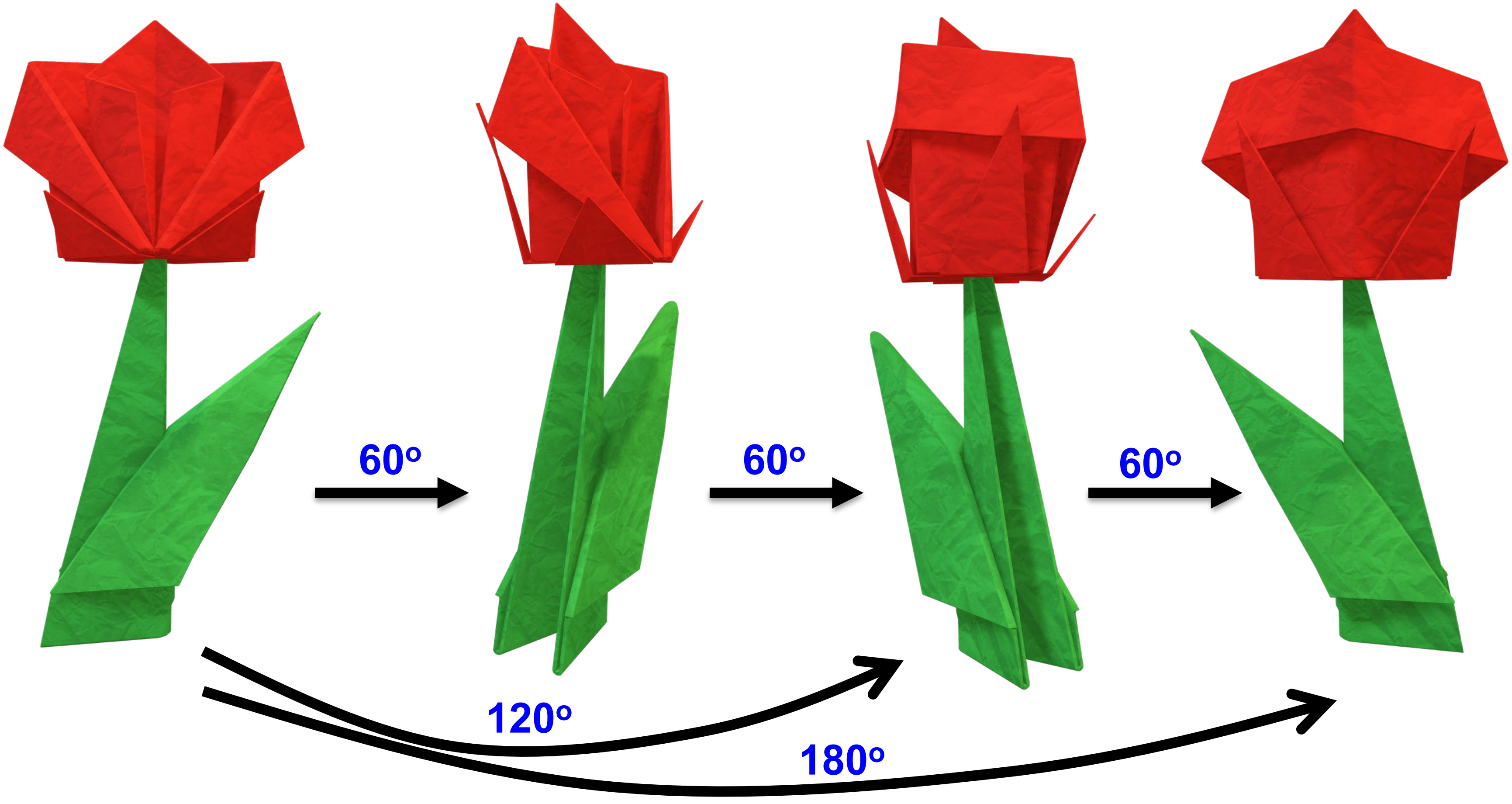
- Natural products produce compounds that can be used in many area (biomaterials, textile polymers,…)

- Natural products produce compounds that can be used to understand pharmacological mechanisms (Capsaicin Nobel winner 2021)

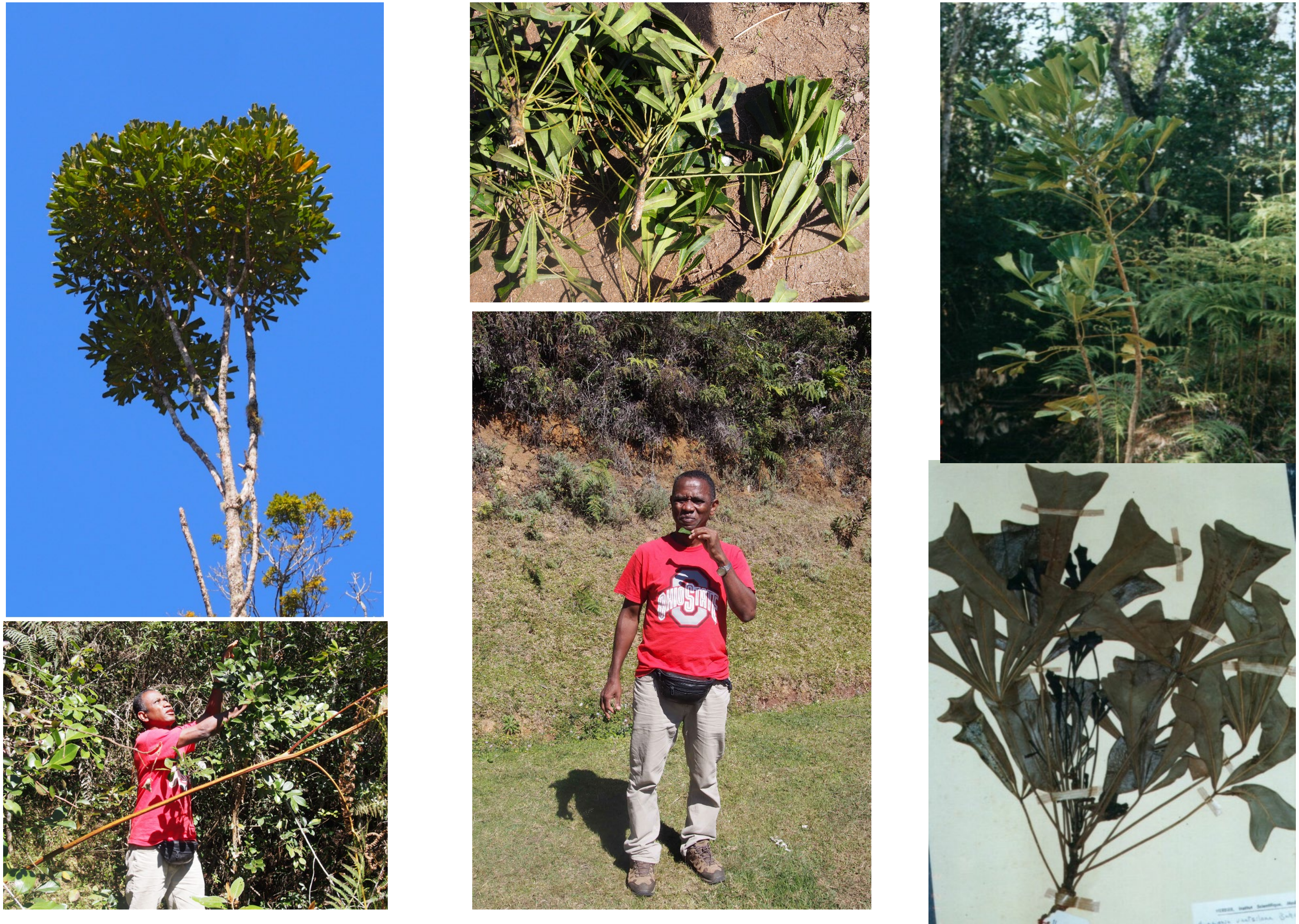
# Making Pieces of the Challenging Puzzles of Natural Product Research


Green
White
Orange

# Understanding the Problems and Challenges

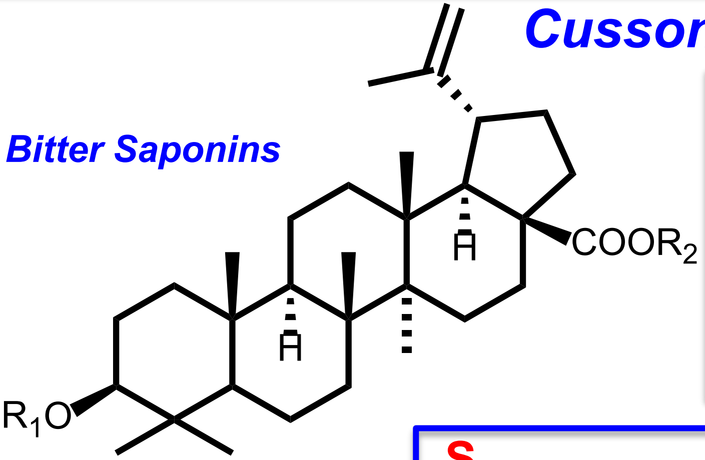

60°
60°
60°
120°
180°

# *Cussonia vantsilana* (Araliaceae) endemic to Madagascar



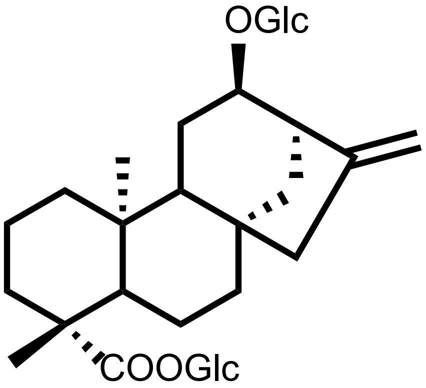
# *Cussonia racemosa and C. vantsilana* (Araliaceae)

***Bitter Saponins***


Bitter Saponins
H
H
COOR$_2$
R$_1$O

| | R$_1$ | R$_2$ |
|---|---|---|
| 1: | S$_2$ | S$_4$ |
| 2: | S$_3$ | S$_4$ |
| 3: | H | H |
| 4: | S$_1$ | S$_5$ |
| 5: | S$_1$ | S$_6$ |

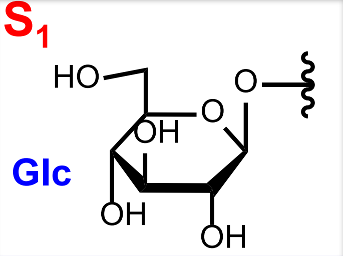
***Sweet diterpene glucoside***

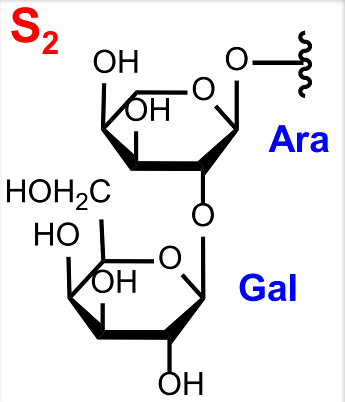

OGlc
COOGlc

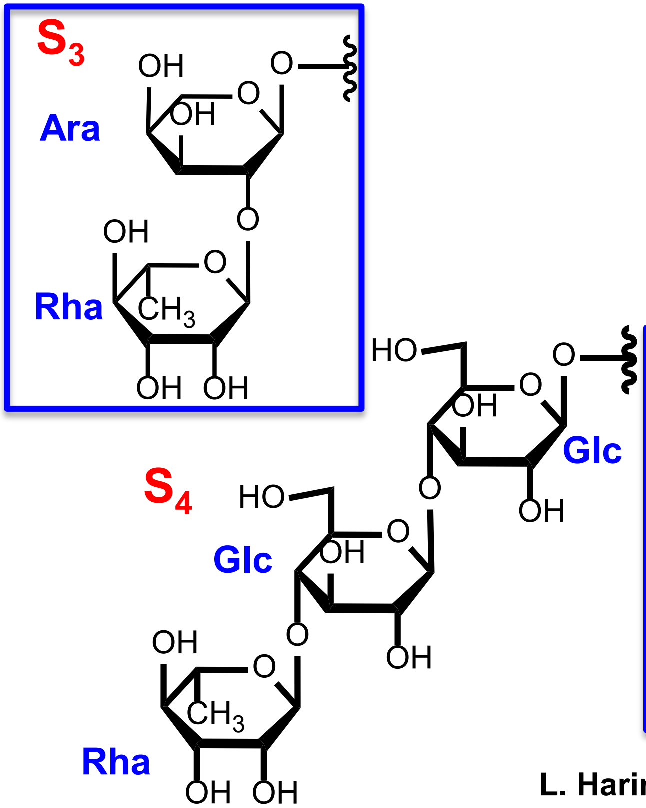
**Cussoracoside C**

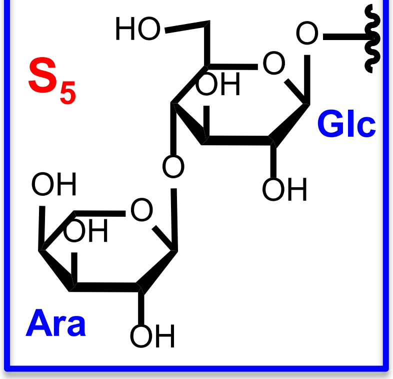
**13 times as sweet as sucrose (0.1% yield w/w)**

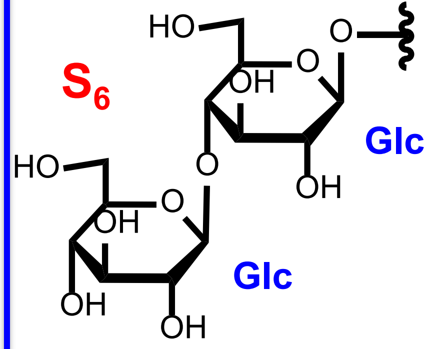
***Sweetness relative to sucrose, based on weight**


S$_1$
HO
O
O
OH
Glc
OH
OH


S$_2$
OH
O
O
OH
Ara
HOH$_2$C
O
HO
O
OH
Gal
OH

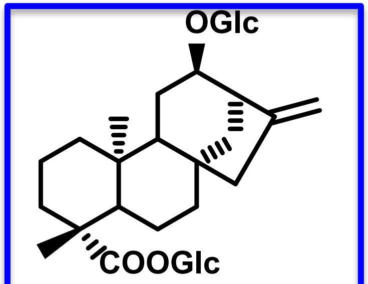

S$_3$
OH
O
Ara
O
OH
O
OH
O
Rha
CH$_3$
OH
OH
HO
O
O
OH
Glc
S$_4$
HO
O
OH
O
Glc
OH
OH
O
OH
O
CH$_3$
Rha
OH
OH


HO
O
S$_5$
O
OH
Glc
O
OH
OH
O
OH
Ara
OH


HO
O
O
S$_6$
OH
Glc
HO
O
OH
O
OH
Glc
OH
OH

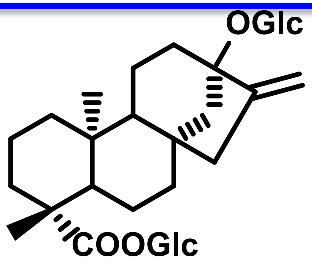
L. Harinantenaina, *Chem. Pharm. Bull.* 50, 1290 (2002)

# Sweet *ent*-kaurene glucosides


OGlc
COOGlc

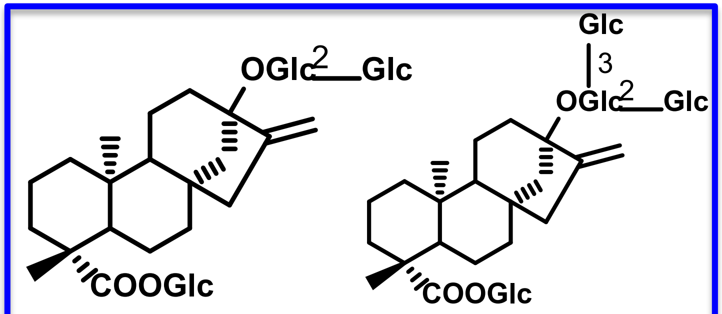
**Cussoracoside C**

Source: *C. racemosa* (Araliaceae), 13 times as sweet as sucrose*


OGlc
COOGlc

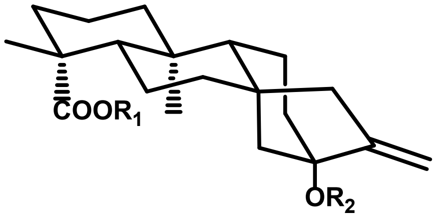
**Rubusoside**

Source: *Rubus souavissimus* (Rosaceae)


OGlc
2
Glc
COOGlc
Glc
3
OGlc
2
Glc
COOGlc

**Stevioside** **Rebaudioside A**

Source: *Stevia rebaudiana* (Asteraceae)



| Glycoside | $R_1$ | $R_2$ | RS* |
|---|---|---|---|
| Stevioside | -Glc | -Glc-(2-1)-Glc | 143 |
| Rebaudioside A | -Glc | Glc-(2-1)-Glc<br>(3-1)-Glc | 242 |
| Rebaudioside D | -Glc-(2-1)-Glc | Glc-(2-1)-Glc<br>(3-1)-Glc | 221 |
| Rebaudioside E | -Glc-(2-1)-Glc | -Glc-(2-1)-Glc | 174 |
| Rubusoside | -Glc | -Glc | 114 |
| Cussoracoside C | | | 13 |

Tanaka, O. *Pure & Appl. Chem.* 69, 675-683 (1997)

* Sweetness relative to sucrose, based on weight

# Natural product compounds have many applications

Knowing the chemical landscape of natural products helps to build new research topics.

How?

**Untargeted metabolomics and/or isolation and structure elucidation of secondary metabolites.**

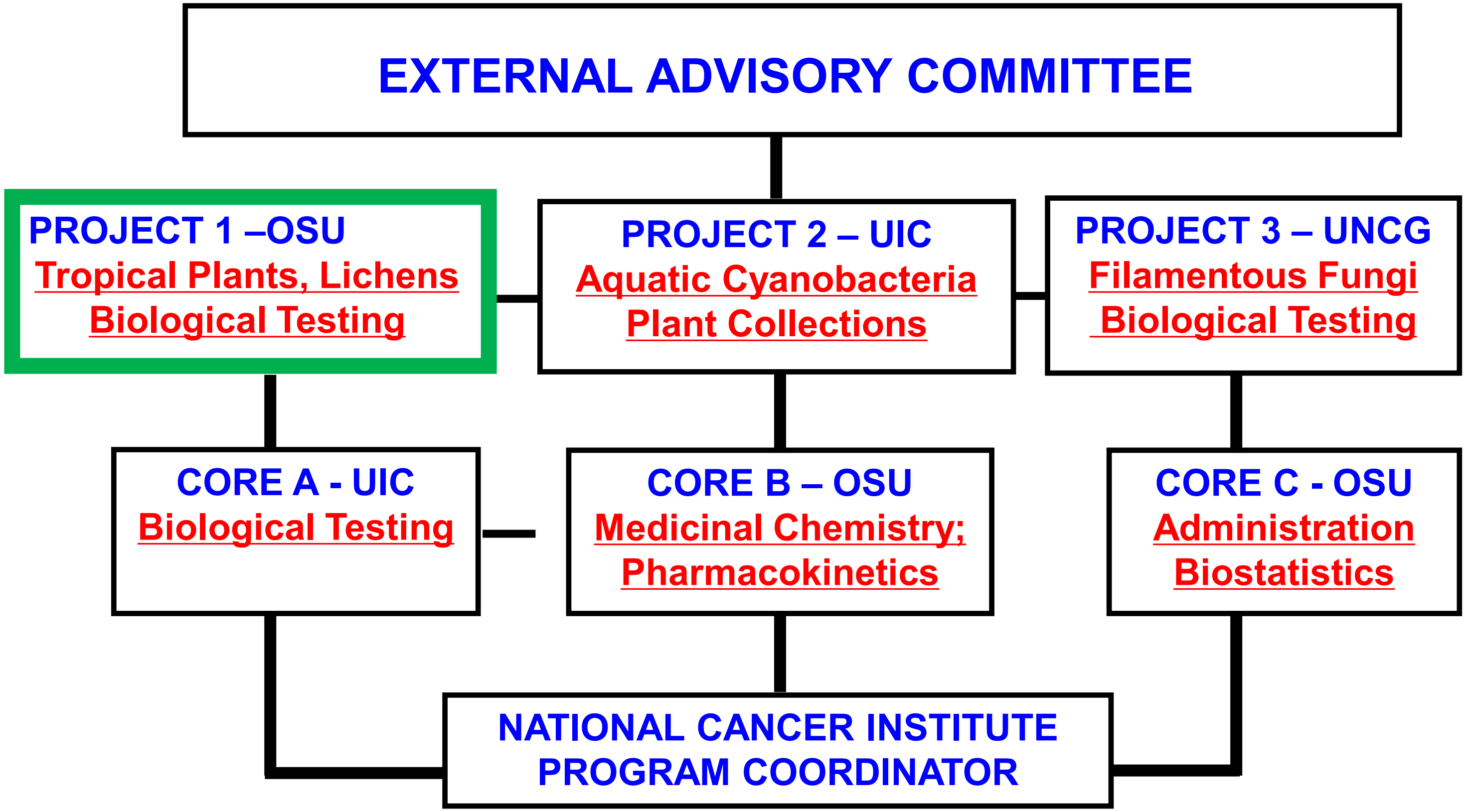
↓

**Identification of possible hit compounds: Screening or use of databases and published research data. Then validation**

↓

**Medicinal chemistry and bioengineering studies to obtain lead compounds**

# P01 PROJECT CA125066 - A MULTIDISCIPLINARY AND MULTI-INSTITUTIONAL APPROACH (2007-2025)



EXTERNAL ADVISORY COMMITTEE

PROJECT 1 –OSU
Tropical Plants, Lichens
Biological Testing

PROJECT 2 – UIC
Aquatic Cyanobacteria
Plant Collections

PROJECT 3 – UNCG
Filamentous Fungi
Biological Testing

CORE A - UIC
Biological Testing

CORE B – OSU
Medicinal Chemistry;
Pharmacokinetics

CORE C - OSU
Administration
Biostatistics

NATIONAL CANCER INSTITUTE
PROGRAM COORDINATOR

**3P01 CA125066-09S1 (10/2017~ 04/2019):**

**NEW SOURCES OF ANTICANCER DRUGS: ENDEMIC COASTAL MACROLICHENS AND THEIR ASCOMYCETES AND BASIDIOMYCETES MYCOBIONTS**

# DRUG DISCOVERY AND DEVELOPMENT TEAM

**"Discovery of Anticancer Agents of Diverse Natural Origin"**
**(NCI/NIH – P01CA125066; Program Project)**

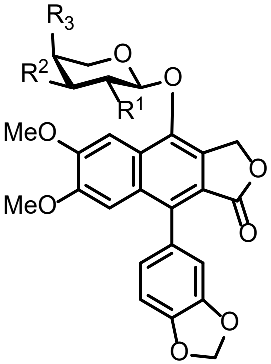
**Serves as a multi-institutional discovery and development platform for natural products**

***Project 1:* Douglas Kinghorn, OSU (plants/lichens)**
***Project 2:* Jimmy Orjala, UIC (cyanobacteria)**
***Project 3:* Nicholas Oberlies, UNC-G (fungi)**
***Core 1:* Joanna Burdette, UIC (biological assays)**
***Core 2:* James Fuchs, OSU (Med. Chem./PK)**
***Core A:* Administration/Biostatistics, Douglas Kinghorn, OSU**

**Combines expertise in natural products isolation, cancer biology, *in vitro* and *in vivo* bioassays, chemical synthesis, and pharmacokinetics.**

# PHYLLANTHUSMIN LIGNANS (INITIAL PHYTOCHEMICAL ISOLATION) (UIC/OSU)

Photo by D.D. Soejarto



Dr. Doel Soejarto, UIC

Dr. Yulin Ren, OSU

- *Phyllanthus poilanei* (Phyllanthaceae) samples were collected in Vietnam by Dr. Doel Soejarto (UIC).
- Using a bioassay-guided fractionation approach (HT-29 colon carcinoma cells), Dr. Yulin Ren (OSU) isolated two new and four known arylnaphthalene (phyllanthusmin) lignan lactones from the chloroform-soluble extract (general structure shown above).
- Phyllanthusmin D (new; $R_1$ = OH; $R_2$ = $R_3$ = OAc) exhibited micromolar activity against HT-29 human colon cancer cells.
- Isolated phyllanthusmin D was active in an *in vivo* hollow fiber assay (with immunodeficient NCr *nu*/*nu* mice; 10-20 mg/kg ip) (UIC).

(Ren *et al.*, *J. Nat. Prod. 77*, 1494, 2014)

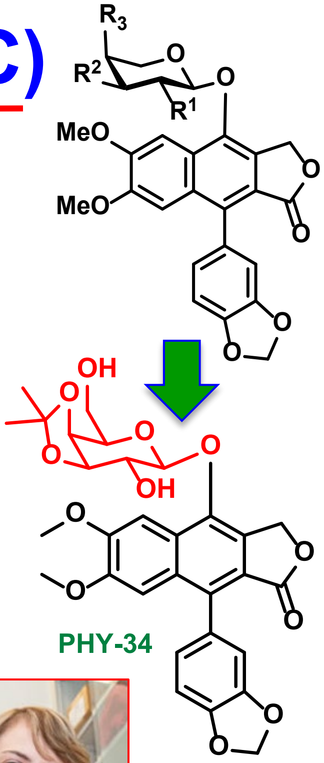
# PHYLLANTHUSMIN LIGNANS (OPTIMIZATION BY CHEMICAL SYNTHESIS; OSU/UIC)

- A chemical synthesis program to generate phyllanthusmin derivatives with favorable biological and physicochemical properties was conducted (Dr. James Fuchs; OSU).

- A key chemically synthesized lead compound, PHY-34, was found to be much more potently cytotoxic than phyllanthusmin D [IC$_{50}$ values of 43, 4, and 4 nM for the HT-29 (colon), OVCAR3 (ovarian) and OVCAR8 (ovarian) cell lines, respectively].

- In a biological follow-up study conducted at UIC (Dr. Joanna Burdette), PHY-34 was found to act as a late-stage autophagy inhibitor, and to have significant antitumor efficacy as a single agent against high-grade serous ovarian cancer *in vivo*.

**Dr. James Fuchs, OSU**



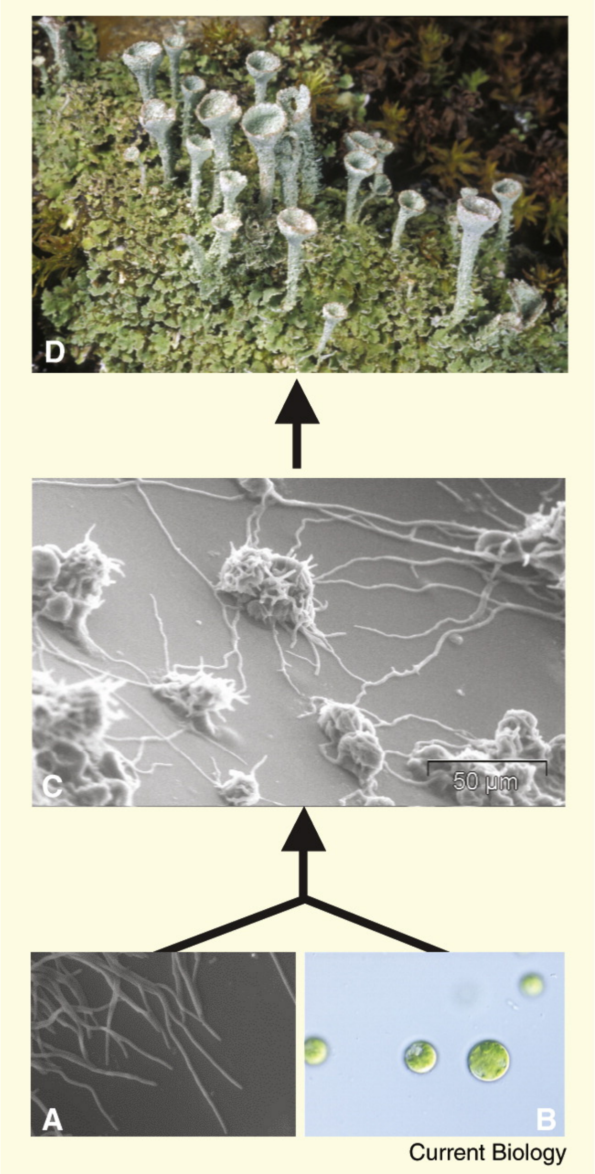
PHY-34

**Dr. Joanna Burdette, UIC**

(Woodard *et al*., *Bioorg. Med. Chem*. *26*, 2354, 2018; Young *et al*., *Mol. Cancer Ther*. *17*, 2123, 2018)

# Lichens and their mycobionts for cytotoxic compounds

**Rakotondraibe Lab**



(D) Mature lichen in nature resulting from the association

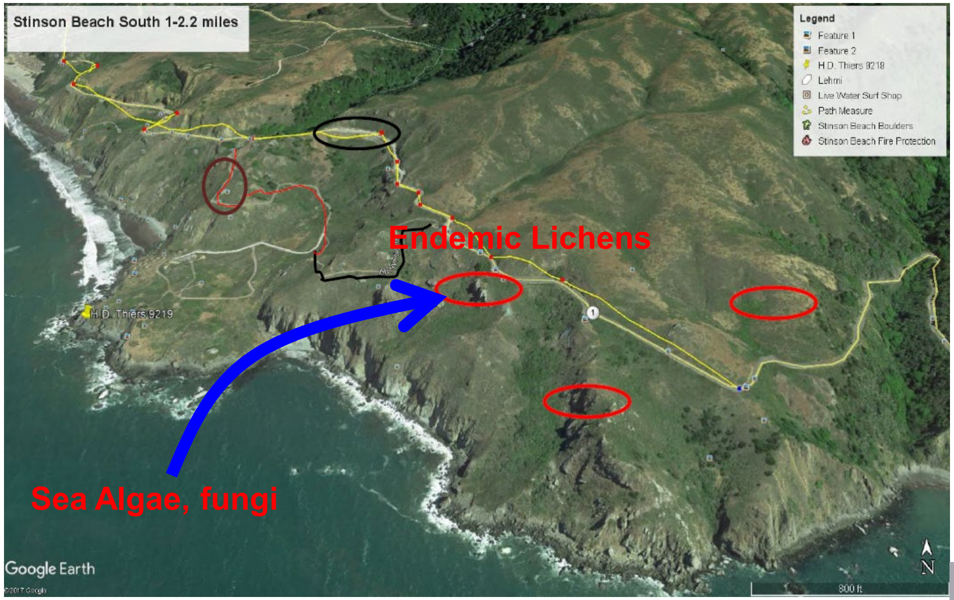
(C) *Cladonia grayi* growing with *Asterochloris* sp. in culture clumps of algal cells overgrown by branching mycobiont and connected by mycobiont hyphae of *Cladonia grayi* with *Asterochloris* sp.
(photo: Stephen Sharnoff).

(A) Mycobiont *Cladonia grayi* growing separately in culture

(B) Unicellular photobiont *Asterochloris phycobiontica* growing separately in culture

Lutzoni, F. and Miadlikowska, J. 2009, *Current Biology*, 19, R502-R503DOI: (10.1016/j.cub.2009.04.034)

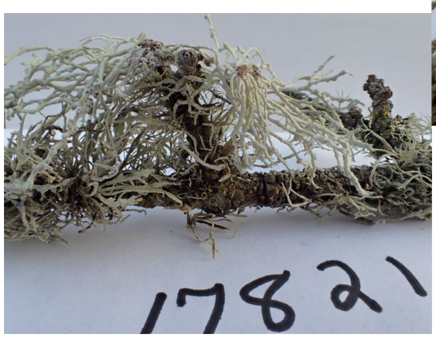
# U.S. endemic lichens


Stinson Beach South 1-2.2 miles
Legend
Feature 1
Feature 2
H.D. Thiers 8218
Lehmi
Live Water Surf Shop
Path Measure
Stinson Beach Boulders
Stinson Beach Fire Protection
Endemic Lichens
H.D. Thiers 9219
Sea Algae, fungi
Google Earth
800 ft
N

○ *Vermilacinia* species
○ *Niebla* species

***Collection map provided by Dr. R. Spjut***
World Botanical Associates

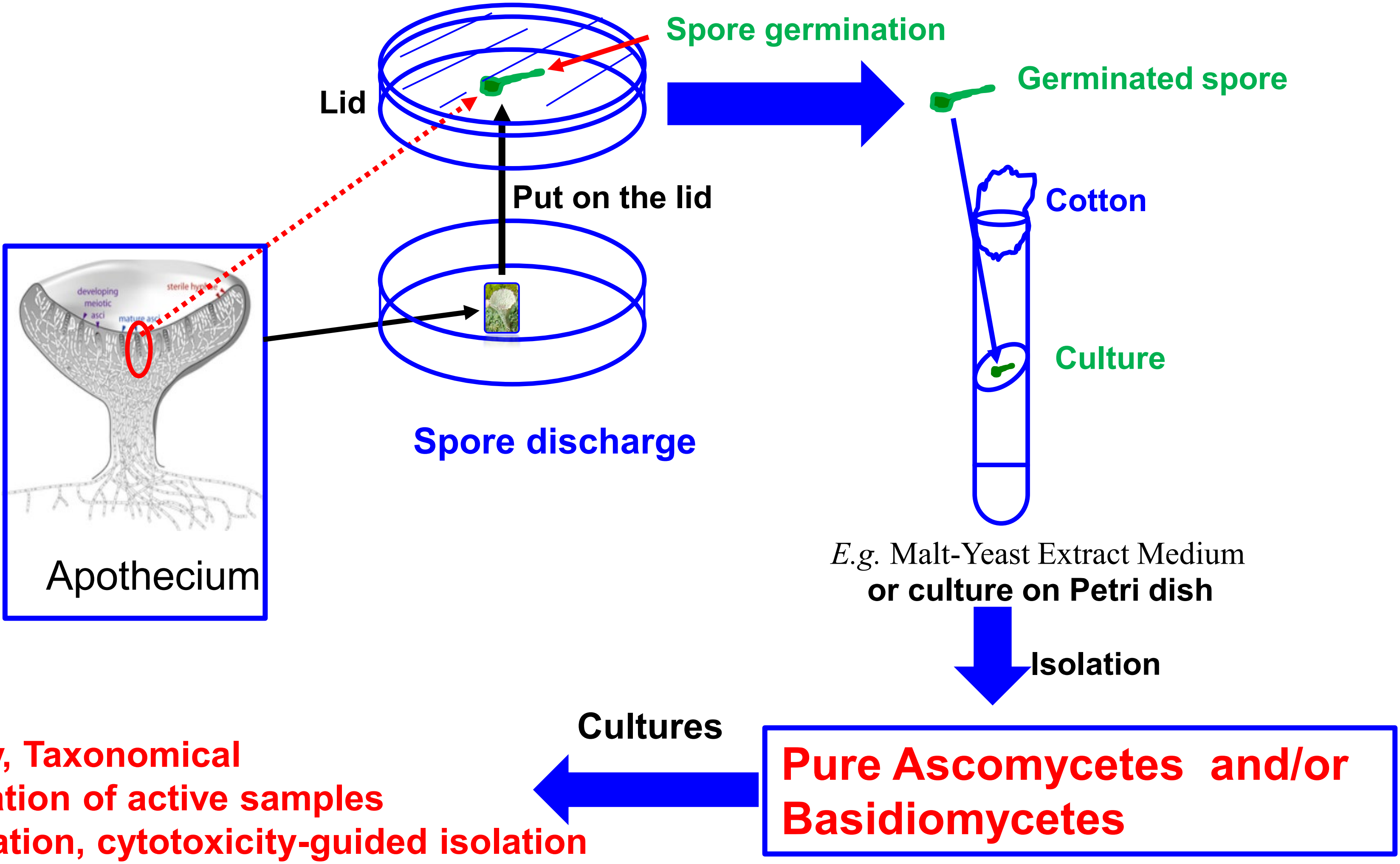
## Pictures of lichens



**Lichen species of the genus *Niebla***

# Isolation of lichen mycobionts

**Example of lichen**



Spore germination
Germinated spore
Lid
Put on the lid
Cotton
developing meiotic asci
sterile hyphae
mature asci
Culture
Spore discharge
Apothecium

*E.g.* Malt-Yeast Extract Medium
**or culture on Petri dish**

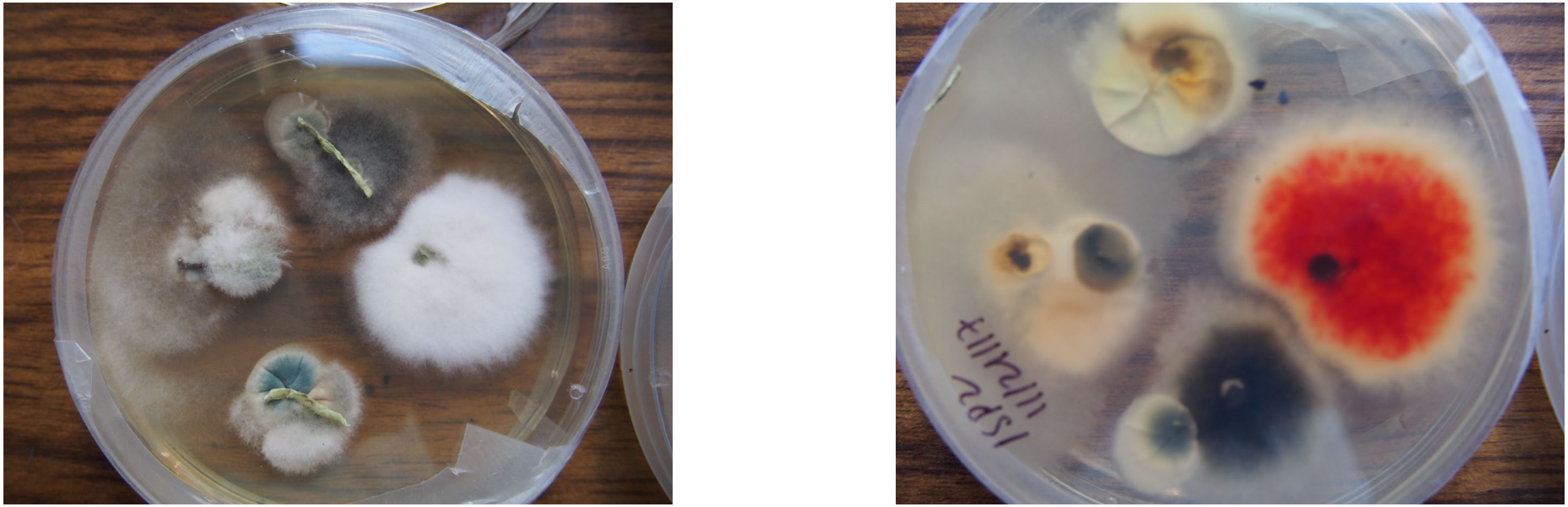
**Isolation**

**Cultures**

**Pure Ascomycetes and/or Basidiomycetes**

- **Library**
- **Bioassay, Taxonomical identification of active samples**
- **Dereplication, cytotoxicity-guided isolation**
- **Understanding the chemical landscape**

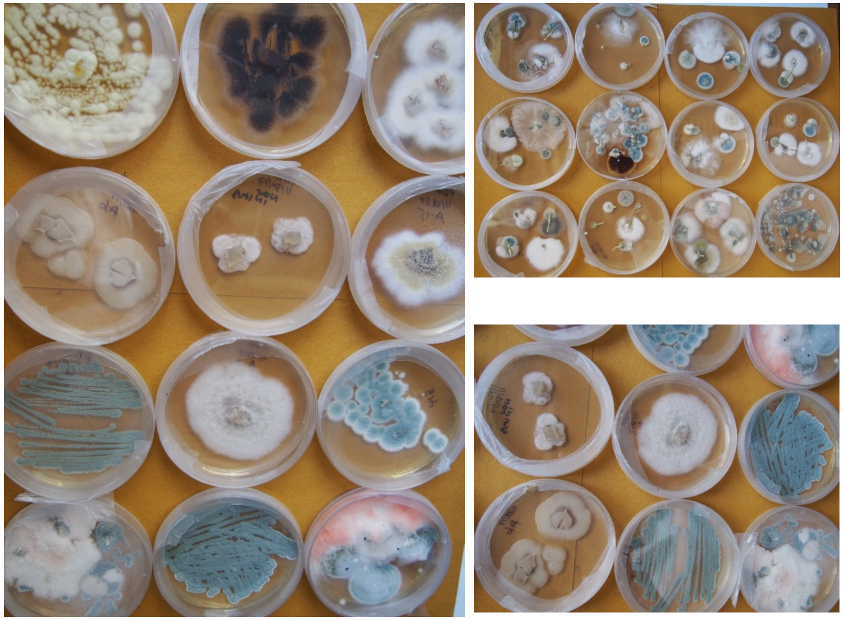
# Examples of lichen associated fungi obtained



- **150 Lichen samples have been received from Dr. R. SPJUT (World botanical Associates)**
- **Different collections of the U.S. endemic *Niebla homalea* were selected for further investigation**

***Niebla homalea* (Ramalinaceae)**

**Representatives of the Isolated fungi**



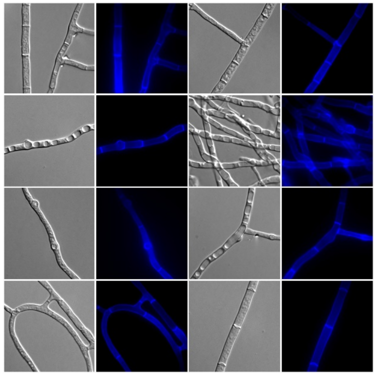
| Sample | A2780 [a]IC$_{50}$ | MCF-7 [a]IC$_{50}$ | HT-29 [b]Surv. | MDA-MB-231, [b]Surv. |
|---|---|---|---|---|
| Control | 0.001[P] | 0.002[C] | 0.001 | |
| RAK_A1 | <0.16 | <0.16 | 4 | NT |
| RAK_A2_1 | >20 | 18.5 | NT | NT |
| RAK_A3 | >20 | 18.1 | NT | NT |
| RAK_A4 | >20 | >20 | NT | NT |
| RAK_A5 | 2.9 | 3.1 | [b]61% | 78% |
| RAK_A6 | >20 | 17.3 | NT | NT |
| RAK_A8 | 0.5 | 0.3 | NT | NT |
| RAK_A9 | 14.7 | >20 | NT | NT |
| RAK_A15_RE | 0.7 | 0.9 | [b]100% | 100% |
| RAK_A16 | 2.2 | 1.8 | 68% | 100% |
| RAK_A0 | 0.3 | 1.2 | [b]39% | 36% |
| RAK_A17ISP2 | 19.4 | 8.2 | NT | NT |
| RAK_AD | >20 | 17.6 | NT | NT |

[a]IC$_{50}$ in μg/mL; [P]: Paclitaxel; [C]: Camptothecin; [b]: Survival at 20 μg/mL; NT: Not Tested

**RAK_A16 and RAK_A0 were first selected**

**We focused our work on the bioassay-guided isolation of cytotoxic compounds of two fungal associates of the lichen *N. homalea*: RAK-A16: *Penicillium aurantiacobrunneum* and RAK-A0: *Epicoccum nigrum***

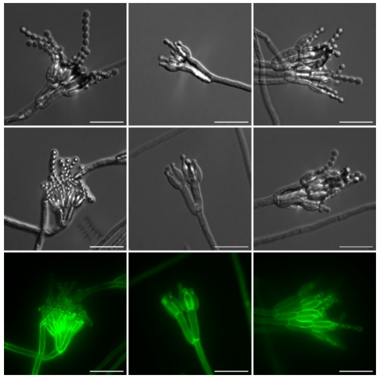
### *Epicoccum nigrum*
### (RAK_AO)



**Conidophores**

**A2780: $IC_{50}$ 0.3 μg/mL**
**MCF7: $IC_{50}$ 1.2 μg/mL**

### *Penicillium aurantiacobrunneum*
### (RAK_A16)



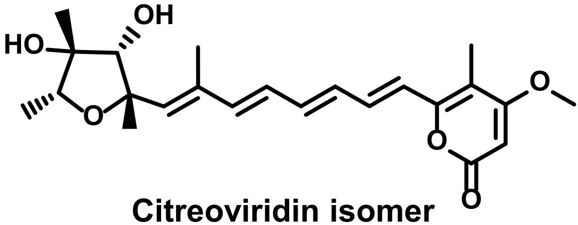
**Conidophores**

**A2780: $IC_{50}$ 2.2 μg/mL**
**MCF7: $IC_{50}$ 1.8 μg/mL**

# A total of 8 compounds, of which 6 are new and 2 are known have been isolated and identified from RAK-A16

## α-pyrone polyene

**Cultured on Potato Dextrose Agar**



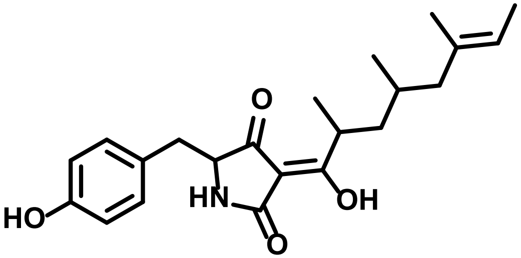
Citreoviridin isomer

| | A2780 | MCF-7 | HT-29 | HeLa | DU-145 |
|---|---|---|---|---|---|
| $IC_{50}$ (μM): | 5.7 | 4.2 | >10 | >10 | >101.5 |

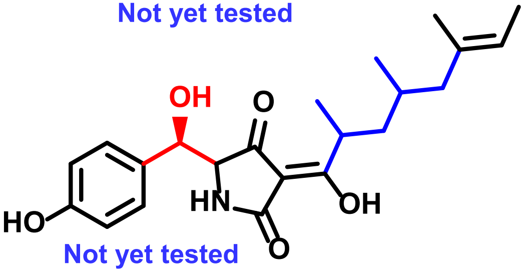
C.Y. Tan, Rakotondraibe *et al.* J. Nat. Prod. 2019, 82, 9, 2529–2536.

## Tetramic acids

**Cultured on Brown Rice**

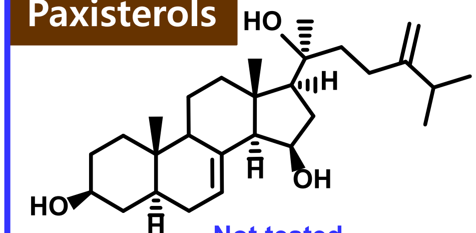


Not yet tested



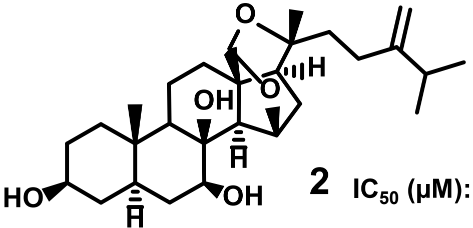
Not yet tested

## Paxisterols



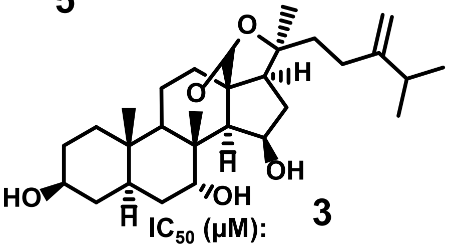
5

Not tested



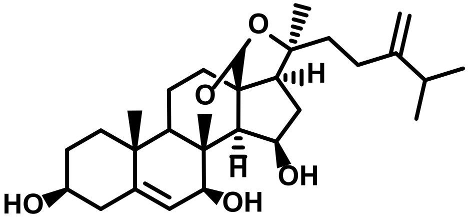
2

| | | | | | |
|---|---|---|---|---|---|
| $IC_{50}$ (μM): | > 10 | >10 | >10 | >10 | >10 |



3

| | | | | | |
|---|---|---|---|---|---|
| $IC_{50}$ (μM): | > 10 | >10 | 9.8 | >10 | >10 |



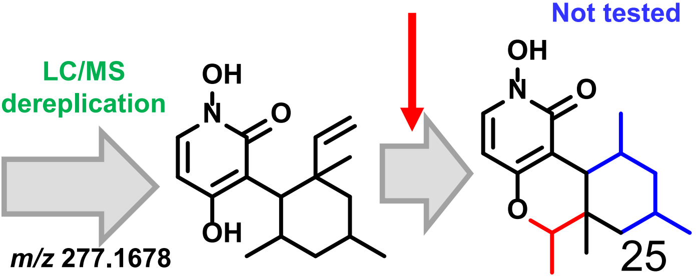
4

Not tested

## Pyridone alkaloids

**Cultured on MEM (Eagle)**

GIBCO
MEM (Eagle)



LC/MS dereplication

*m/z* 277.1678

8-Methyl-pyridoxatin

1D NMR dereplication Isolation

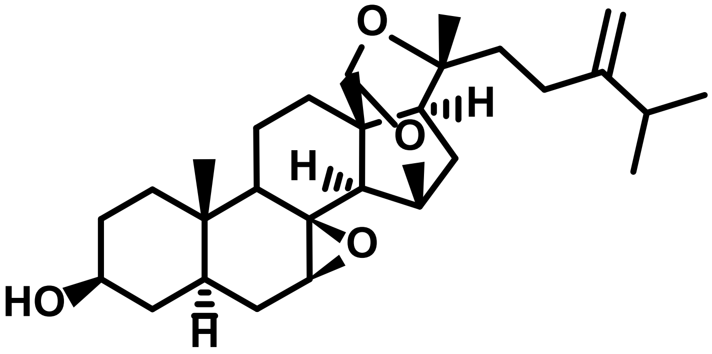
Not tested

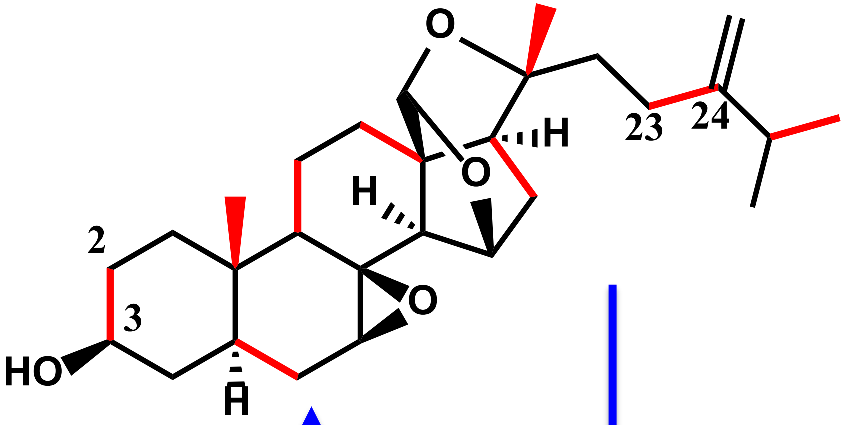
*N*-hydroxy-2-pyridone

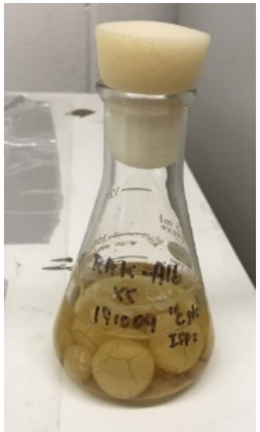
# Understanding the biosynthesis of *P. aurantiacobrunneum (RAK-16)* metabolites for production of bioactive compounds

**Dr. Yoshi Yamano**

## Paxisterol has been isolated as major compound




O
H
23
24
H
O
2
O
3
HO
H


RAK-A16
XS
191009
ISP2

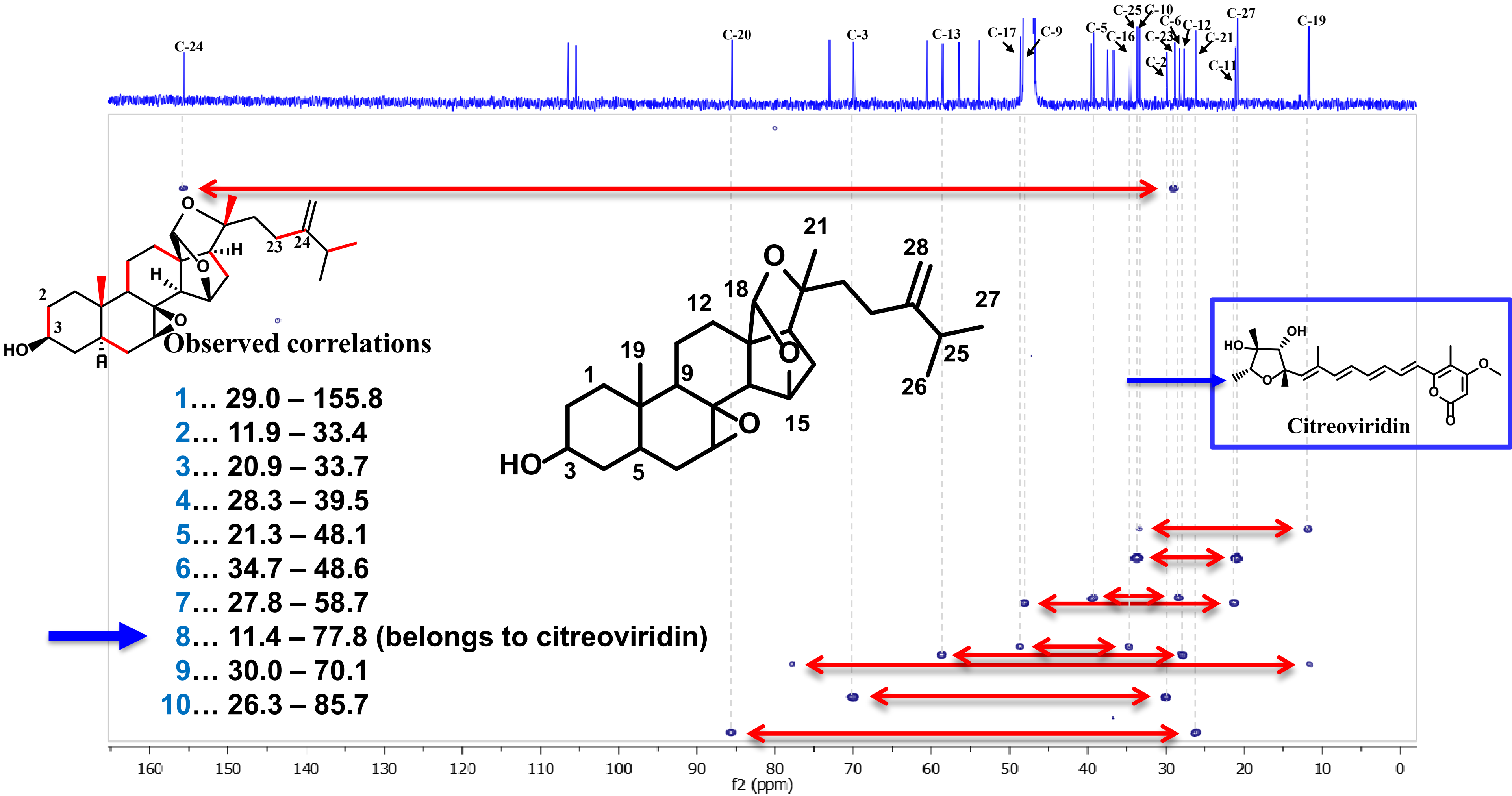
RAK-A16 in ISP2 with $^{13}C_6$-Glucose for 14 days

*Extracted with EtOAc* → *Partitioned with EtOAc*

**EtOAc Extract** → *Isolation* → **Labelled paxisterol**

*Confirmed with reported data*

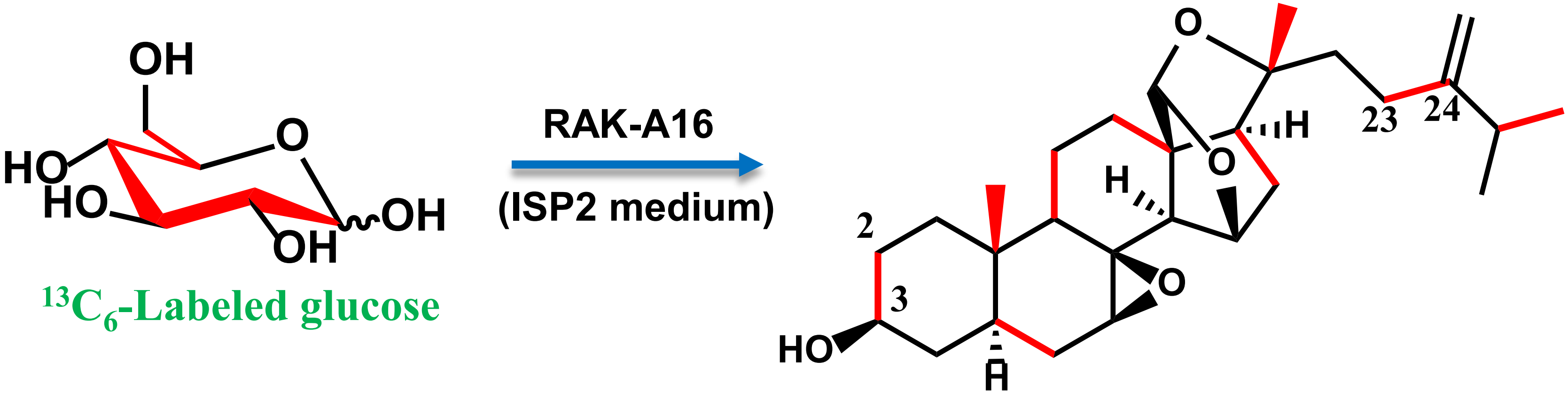
# INADEQUATE Spectrum of the Produced $^{13}$C-labeled Paxisterol



Observed correlations

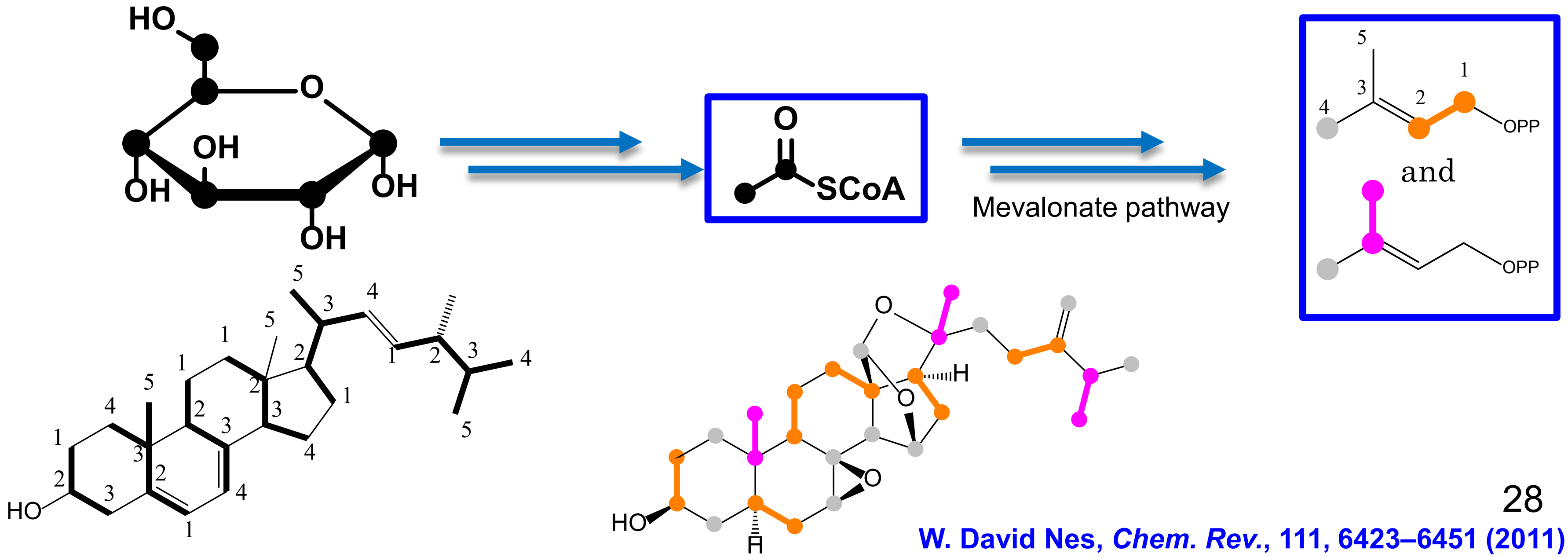
1… 29.0 – 155.8
2… 11.9 – 33.4
3… 20.9 – 33.7
4… 28.3 – 39.5
5… 21.3 – 48.1
6… 34.7 – 48.6
7… 27.8 – 58.7
8… 11.4 – 77.8 (belongs to citreoviridin)
9… 30.0 – 70.1
10… 26.3 – 85.7

# $^{13}$C-labeled Paxisterol from $^{13}$C-labeled glucose


OH
HO
HO
O
OH
OH
RAK-A16
(ISP2 medium)
O
H
24
23
O
H
2
3
O
HO
H

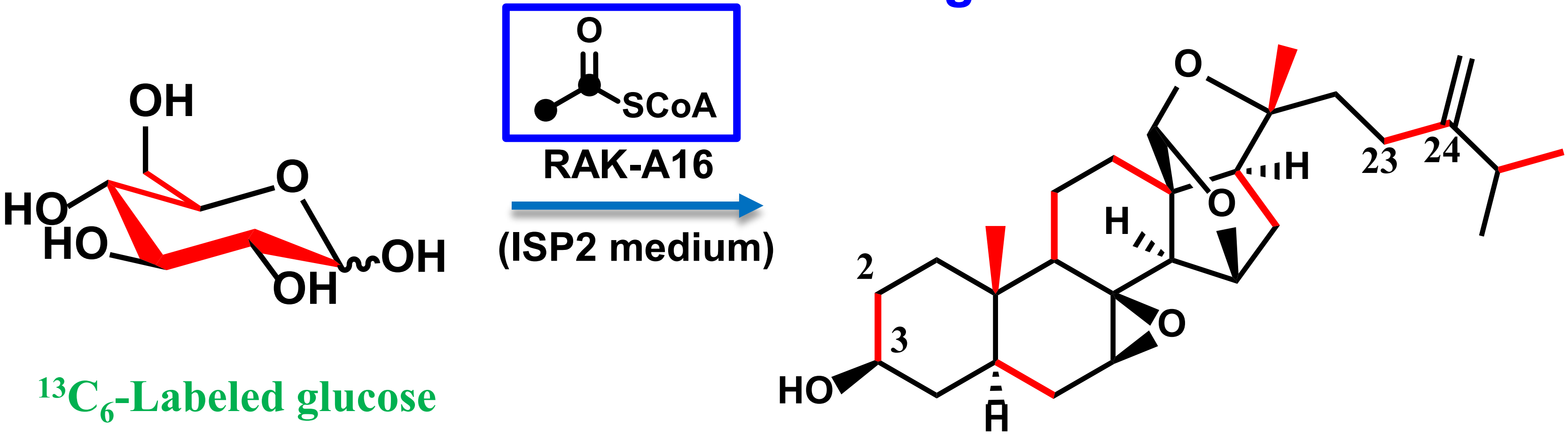
$^{13}C_6$-Labeled glucose

# Interpretation and comparison with reported data


HO
O
OH
OH
OH
OH
O
SCoA
Mevalonate pathway
5
1
3
2
4
OPP
and
OPP
5
3
4
1
2
5
1
2
3
1
2
1
4
5
1
2
3
2
3
4
5
4
1
3
2
1
2
3
HO
O
H
O
O
HO
H

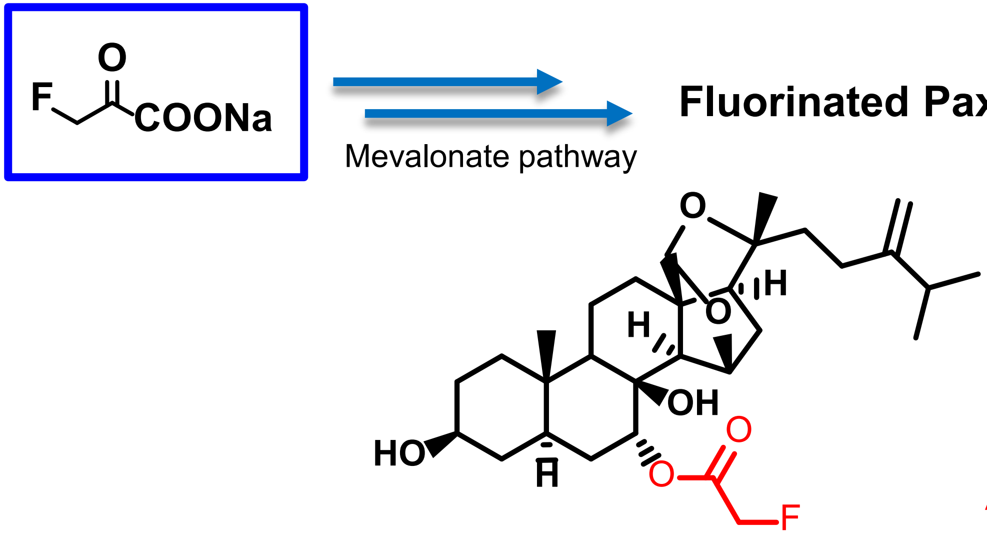
W. David Nes, *Chem. Rev.*, 111, 6423–6451 (2011)

## $^{13}$C-labeled Paxisterol from $^{13}$C-labeled glucose


OH
HO
HO
O
OH
OH
O
SCoA
RAK-A16
(ISP2 medium)
O
O
H
H
O
2
3
HO
H
23
24

$^{13}C_6$-Labeled glucose

## Production of halogenated derivative(s)

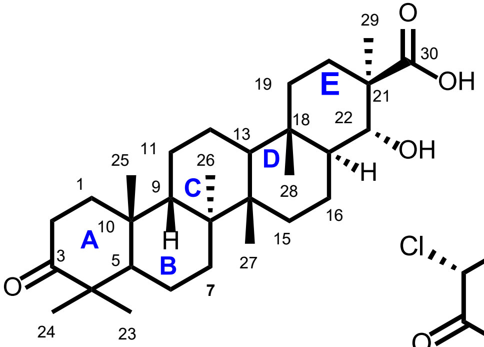

O
F
COONa
Mevalonate pathway
O
H
O
H
OH
HO
H
O
O
F

**Fluorinated Paxisterol?**

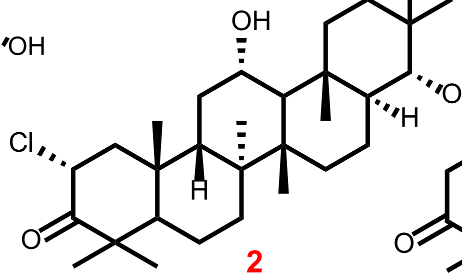
**Abiotic?**

***Niebla homalea***
**(An U.S. endemic Lichen)**

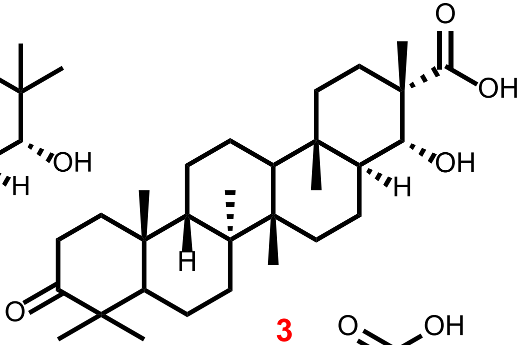
# New and Known Compounds from *Niebla homalea*


O
OH
OH
H
H
A
B
C
D
E
1
3
5
7
9
10
11
13
15
16
18
19
21
22
23
24
25
26
27
28
29
30

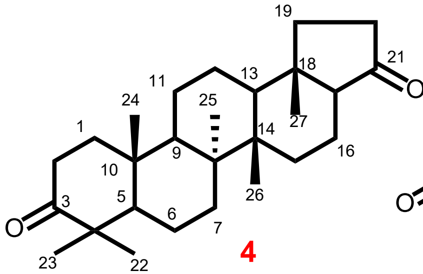
1



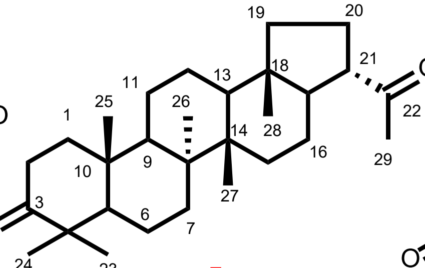
2



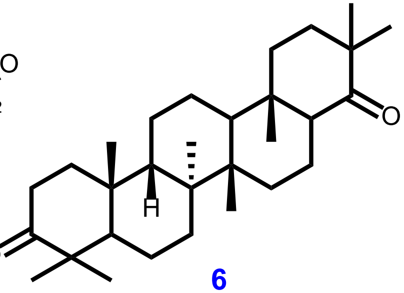
3


O
O
1
3
5
6
7
9
10
11
13
14
16
18
19
21
22
23
24
25
26
27

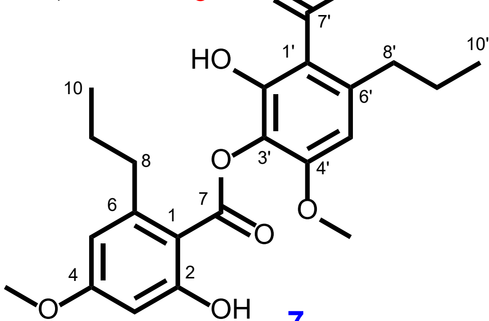
4


O
O
1
3
6
7
9
10
11
13
14
16
18
19
20
21
22
23
24
25
26
27
28
29

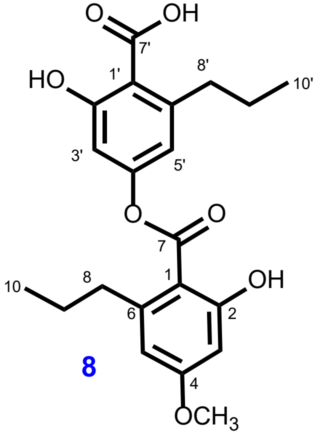
5



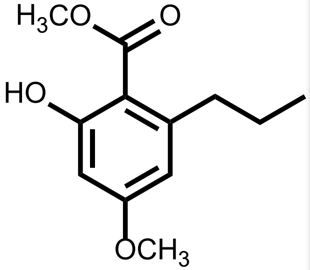
6


O
OH
HO
O
O
O
O
OH
1'
3'
4'
6'
7'
8'
10'
1
2
4
6
7
8
10

7


O
OH
HO
O
O
OH
OCH3
1'
3'
5'
7'
8'
10'
1
2
4
6
7
8
10

8


H3CO
O
HO
OCH3

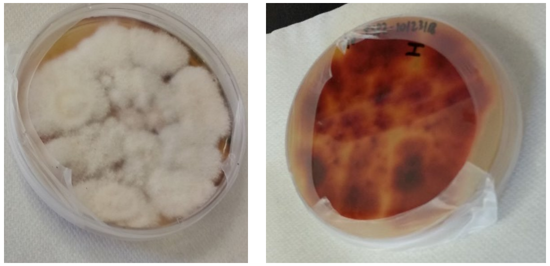
9

- Compound **2** showed weak antiproliferative activity against breast (MCF-7) and ovarian (A2780) cancer cell lines.
- Compound **3** is selective toward MDA-MB-231, triple negative breast cancer cell line.
- All compounds will be evaluated for activity against other cancer cells available in the program

**Dr. Yan Zhang**

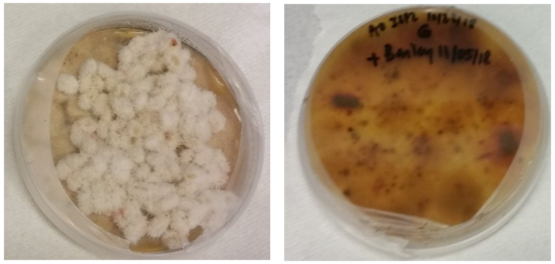
# Various Fermentations of *Epicoccum nigrum* (A0) and their cytotoxic activity

**A0 on ISP2 Agar**

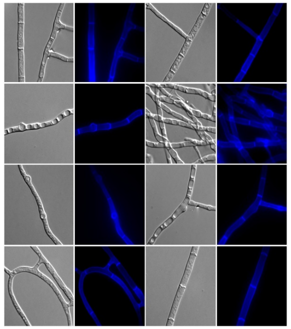


Top
Bottom

**A0 on ISP2 Agar + Barley**



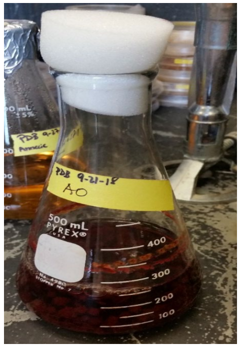
Top
Bottom



**Conidiophores**

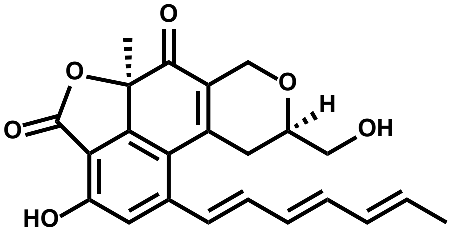
| Extracts | IC$_{50}$ (µg/mL) | |
|---|---|---|
| | A2780 | MCF-7 |
| **A0-PDB-092118-7d-EA** | > 20 | NT |
| **A0-PDB-092118-7d-W** | > 20 | NT |
| **A0-ISP2-101118_7d-EA** | 3.0 | 5.3 |
| **A0-ISP2-110518_9d-EA** | 1.9 | 1.4 |
| **A0-ISP2-110518_9d-M** | > 20 | > 20 |
| **A0-ISP2-broth-101618-7d-EA** | > 20 | > 20 |
| **A0-Barley-110518-8d** | > 20 | > 20 |
| **A0-ISP2+Barley-110518-9d-EA** | 13.4 | 13.2 |
| **A0-ISP2+Barley-110518-9d-M** | > 20 | > 20 |
| **A0-BrownRice-031419-11d-EA** | > 20 | > 20 |
| **Paclitaxel (control)** | 0.005 | |
| **4-hydroxytamoxifen (control)** | | 7.8 |

**A0 in PDB**



**A0 on Barley**

**A0 on Brown Rice**



**Acetosellin**
**A0-ISP2-101118_7d-EA**

# SUMMARY

- **New and cytotoxic compounds have been isolated from the lichen *Niebla homalea* and its fungal associates.**
- **Understanding the chemical landscape of natural products can lead to new topics in natural product research.**
- **Supplementation of modified (halogenated) precursors in fungal cultures led to the production of new and/or bioactive compounds.**
- **Dereplication methods (quick identification of known and promiscuous compounds) are needed to lead the discovery of new bioactive compounds.**

# Acknowledgments

**Collaborators:**

- **Dr. Richard Spjut (World Botanical Associates, Bakersfield, CA)**
- **Dr. Chad Rappleye (Department of Microbiology, OSU)**
- **Dr. Joanna E. Burdette (UIC, Medicinal Chemistry and Pharmacognosy)**
- **Dr. Nicholas Oberlies (UNC Greensboro, NC)**
- **Dr. A. Douglas Kinghorn (OSU, College of Pharmacy)**
- **Dr. Doel Soejarto (UIC, Medicinal Chemistry and Pharmacognosy)**

**Lab members:**

- **Dr. Annecie Benatrehina**
- **Dr. Yan Zhang**
- **Dr. Dahai He**
- **Dr. Preston Manwill**
- **Dr. Choon Yong Tan**

**PROGRAM PROJECT:** 5P01 CA125066-10
**Diversity Supplement:** 3P01CA125066-10S1

NIH NATIONAL CANCER INSTITUTE

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American Cancer Society®

NIH National Institute of Allergy and Infectious Diseases

**2003:** PhD from Hiroshima University, School of Medicine, Faculty of Pharmaceutical Sciences (JAPAN)

**2003-2007:** Postdoctoral research fellow working on the phytochemical investigation and biological activities of Medicinal plants and liverworts (Tokushima Bunri University, JAPAN)

**2007-2009:** Assistant Professor at Hiroshima University (JAPAN), Graduate School of Biomedical Sciences

**2009-2013:** Senior Research Scientist at Virginia Tech, Chemistry Department (ICBG project)

**2013-2019:** College of Pharmacy, Div. Med. Chem. & Pharmacognosy
Assistant Professor, The Ohio State University

**2019-Present:** College of Pharmacy, Div. Med. Chem. & Pharmacognosy
Associate Professor (**with tenure**), The Ohio State University

# Building a Research Team to Challenge

## Forming the Team

What do I want? Get advices from Mentors and Colleagues

Previous experience (who can achieve the task?)

Understand and use the system to get help (mentoring classes,…)

Take the responsibility

## Storming

Caring and motivating

Mission must be communicated very clearly

## Norming

Creation of internal (lab/group) rules

## Performing

Periodic group and/or individual meeting

## Self and Peer-Review Evaluations

Always ask yourself and your peers for evaluations

# QUESTIONS?