出國報告（出國類別：參加國際會議）

赴美國參加「美國生藥學會第四十六屆年會（American Society of Pharmacognosy 46th Annual Meeting）」出國報告

服務機關：行政院衛生署中醫藥委員會
職稱：薦任技士
姓名：陳昭蓉
派赴國家：美國
出國期間：民國 94 年 7 月 23 日至 7 月 27 日
報告日期：民國 94 年 10 月 24 日

出國報告提要

出國報告名稱：美國參加「美國生藥學會第四十六屆年會（American Society of Pharmacognosy 46th Annual Meeting）」

計畫補助/委託機關

行政院衛生署中醫藥委員會

出國計畫主辦機關/聯絡人/電話

行政院衛生署中醫藥委員會/陳昭蓉/（02）2587-2828-215

出國人員姓名/服務機關/單位/職稱/電話

陳昭蓉/行政院衛生署中醫藥委員會/中藥組/技士/(02) 2587-2828-215

出國類別：□1 考察　□2 進修　□3 研究　□4 實習　■5 其他

出國期間：94 年 07 月 23 日至 94 年 07 月 27 日

出國地區：美國（奧勒岡州立大學）

報告日期：94 年 10 月 24 日

分類號/目：J0／綜合（醫藥類）

關鍵詞：

生藥學、天然藥物、中草藥、中藥、ASP

內容摘要：

爲積極推動中藥科技研發了解中醫藥領域之世界趨勢，以配合並融入中醫藥公共政策，也爲了解國際中草藥研究方向及臨床試驗環境，促進新藥研發及產業升級，行政院衛生署中醫藥委員會爲全國最高中醫醫政、中藥藥政最高主管機關，有必要瞭解國際間對天然物產品之研究與開發策略，包括：產品研發、管理政策及法規訂定..等最新資訊，俾便將國際趨勢融入將推動或制定之重要政策中，另更加將我國推動中醫藥相關政績，如：執行迄今已成立 13 家「中藥臨床試驗中心」，並以類似美國 FDA 審核方式通過及核發新藥（中藥）藥證；建立中醫醫院訪查制度；中藥廠全面實施 GMP 以提昇藥品品質；及公告出版台灣首部「臺灣傳統藥典」..等，與國際人士經驗交流。爰派員參加 94 年 7 月 23 日至 27 日爲期 5 天於美國奧勒岡州立大學所舉行之「第 46 屆美國生藥學會年會(The 46th Annual

Meeting of the American Society of Pharmacognosy；ASP）」。本次會議我們邀請的專題演講概括到總主題“有機生物及天然藥物化學的新領域”，包括來自美國、加拿大、亞洲和歐洲等 16 名傑出的講師。其專題演講或討論大都安排於早上，包括天然藥物生物合成、海洋天然物分子藥學、植物新陳代謝工程、合成天然藥物的發展及天然藥物化學的微生物主宿互用。包括 44 個口頭報告，分別在 8 個演講會上進行。7 月 27 日頒獎予來自 Prof. Norman Farnsworth，Illinois, Chicago 指定的頂級 ASP 研究成就。另於 24 日及 26 日安排兩天壁報論文，每天大約有 150 幅之多來自各國未公開發表之研究論文摘要及簡介。主要參加議題包括天然物生物合成、海洋天然物分子藥學、植物新陳代謝工程、合成天然物的發展及研究開發。藉由參與國際會議的機會，瞭解國際朝天然物研究開發藥物之方向，以作爲國內推動中醫藥相關政策之依據與參考。

目　　次

摘 要

爲積極推動中藥科技研發了解中醫藥領域之世界趨勢，以配合並融入中醫藥公共政策，也爲了解國際中草藥研究方向及臨床試驗環境，促進新藥研發及產業升級，行政院衛生署中醫藥委員會爲全國最高中醫醫政、中藥藥政最高主管機關，有必要瞭解國際間對天然物產品之研究與開發策略，包括：產品研發、管理政策及法規訂定..等最新資訊，俾便將國際趨勢融入將推動或制定之重要政策中，另更加將我國推動中醫藥相關政績，如：執行迄今已成立 13 家「中藥臨床試驗中心」，並以類似美國 FDA 審核方式通過及核發新藥（中藥）藥證；建立中醫醫院訪查制度；中藥廠全面實施 GMP 以提昇藥品品質；及公告出版台灣首部「臺灣傳統藥典」..等，與國際人士經驗交流。爰派員參加 94 年 7 月 23 日至 27 日爲期 5 天於美國奧勒岡州立大學所舉行之「第 46 屆美國生藥學會年會(The 46th Annual Meeting of the American Society of Pharmacognosy；ASP）」。本次會議我們邀請的專題演講概括到總主題“有機生物及天然藥物化學的新領域”，包括來自美國、加拿大、亞洲和歐洲等 16 名傑出的講師。其專題演講或討論大都安排於早上，包括天然藥物生物合成、海洋天然物分子藥學、植物新陳代謝工程、合成天然藥物的發展及天然藥物化學的微生物主宿互用。包括 44 個口頭報告，分別在 8 個演講會上進行。7 月 27 日頒獎予來自 Prof. Norman Farnsworth，Illinois, Chicago 指定的頂級 ASP 研究成就。另於 24 日及 26 日安排兩天壁報論文，每天大約有 150 幅之多來自各國未公開發表之研究論文摘要及簡介。主要參加議題包括天然物生物合成、海洋天然物分子藥學、植物新陳代謝工程、合成天然物的發展及研究開發。藉由參與國際會議的機會，瞭解國際朝天然物研究開發藥物之方向，以作爲國內推動中醫藥相關政策之依據與參考。

關鍵詞：生藥學、天然藥物、中草藥、中藥、ASP

壹、目的

本署中醫藥委員會身爲全國中醫藥最高行政主管機關，有必要隨時瞭解時國際研發趨勢，掌握其脈動，俾便將國際趨勢融入將推動或制定之重要政策中。參加此次年會目的乃著重於國外最新生藥學之研究發展，尤其針對中草藥部份收集相關資訊，以了解新禁之知識及潮流，並聯繫相關學者專家，以利研究發展能與國際接軌。

貳、過程

一、行程

本次代表行政院衛生署中醫藥委員會參加，報告人於 94 年 7 月 21 日晚間啓程，於當地時間同日晚間約 20 點 15 分抵達洛杉磯，於當地過夜稍作休息，於 23 日下午轉搭國內班機飛於下午約 7 點左右抵達波特蘭，再轉搭接駁車於晚間約 9 點左右抵達目的地奧勒岡州立大學，隨即至報到處報到並領取相關資料，及參加於晚間開幕酒會，結束後至下榻處 check-in。

本次會議的總主題爲“有機生物及天然物化學的新領域”，包括來自美國、加拿大、亞洲和歐洲等 16 名傑出的講師。包括天然物生物合成、海洋天然物分子藥學、植物新陳代謝工程、合成天然物的發展及天然物化學的微生物主宿互用。共有 44 個研究成果經遴選分別在 8 個演說單元中進行。大會於月 24 日早上陸續開始進行各個主題之專題演說，整個會議於 7 月 27 日晚間 10 日圓滿結束。

二、美國生藥學會（ASP）簡介：

美國生藥學會係由 1923 年設立之植物科學研究所衍生出來，於 1959 年創立，其發展之範圍並非侷限於生藥學，而包含所有與天然物有關的科學。該學會現在有超過 1,100 個成員，Appromimately 學會 40% 的成員爲美國和加拿大以外世界各地的代表超過 60 個國家。

三、會議內容節錄：

由於報告人並非研究領域之專家或學者，尚無法將相關研討內容詳實紀錄，僅能就國外發展研究方向蒐集相關資料俾便作爲制定相關政策之參考。

7 月 24 日早上專題演講，其主題爲 Contemporary Approaches in Natural Products Biosyntheies：

（一）Heinz G. Floss

Floss教授研究著重在自然產物的生化合成, 目前主要在抗生素的化學,生物化學

和分子生物學的合成,並且在立體化學級酵素反應的機制。 他發表超過400份原始文章, 回顧和書的章節。

從麥角胺到 ANSAMYCINS – 在生物合成的45年

45年以前, 作者研究生物合成領域,偶然地, 同時研究了麥角胺的型成。這主題已佔用了 他另外20年,而部份的結果和凸出的發表示被重視的。這項研究的發展分成兩個方向，研究立體化學和酵素反應的機制以及抗生素的生物合成。
在八十年代開始, 放射菌分子生物學上的進步向這一領域中添加了新方向, 它將在抗生素生化合成, rifamycins , antitubercular grugs和 maytansinoids抗腫瘤的藥物上加以運用闡明。

（二）Fumitaka Kudo, Ph.D.

Fumitaka 跟隨這他到美國和參加教授 大衛 E 的實驗室。2003 年 ,他回到日本和東京學院(研究所)kakinuma 教授合作，由於前不久 Fumitaka 已去逝，助手教授開始他的學術論著之整理。包括 aminoglycoside 和 polyketide 的抗生素的天然物的生合成。

2-Deoxystreptamine 近代發展包含 AMINOCYCLITOL 抗生素

含有 aminocyciitol 抗生素的 2-Deoxystreptamine(DOS)是重要的抗生素，包含 kanamycin, neomycin 等。 DOS 的 碳環形的 架構由關鍵生物合成胺 2-eoxy-scyllo-inosose synthase(DOIS)催化的D--glucose-6-phosphate形成。本篇討論已經從 butirosin-producing bacillus circulans 純化 出 DOIS 和確認它的基因 btrC 。 之後說明了 butirosin 生物合成的基因組(btr)。既然不能由簡單相似搜查的方式將大多數 open reading frames(ORFs)有效地確認,本篇研究以想像相關的生物合成的基因組可以爲這些抗生素的核心架構提供就重要的生物合成基因來說重要資訊。 這樣 neomycin 的生物合成的基因組, 藉由 streptomyces fradiae 已經確定了。結果, 以 neo cluster 找到了大多數基因, 而這似乎顯得是核心 ribostamycin 生物合成的原因。

（三）Rolf Müller, Ph. D.

1998-2003 他成爲德國 GBF (德國生物技術研究中心)組組長，帶動黏絲菌次要新陳代謝形成的分子生物學研究。2000 年取得 Technischen Universitat carolo-Wilhelmina, braunschweig 資格。2003 年他重新安置實驗室在 Saarbrucken 大學，現在是該大學藥學生物學技術的教授。她的研究著重於分子及微生物化學原理及異性識別和改善黏絲菌天然產物合成。

天然產物研究：對於自然產物利用細菌的基因體和新的異性的表現技術研究

微生物起源的自然產物經常是利用多種合成酶的生物合成作用行成。這些合成酶的遺傳工程和異性表現提供相當多種用途 ,尤其如果這些自然宿主不容易處理, 成長緩慢, 或者甚至是未知的。Myxobacteria 是一個好例子。 利用廣泛分析 stigmatella aurantiaca 基因可能性來生成 polyketide 的生物活性物質或者 nanribosomal 的 胜肽,它證明能夠由基因遮蔽方法發現新的二級代謝物。分析 myxococcus xanthus 和 sorangium cellulosum 的基因組序列近一步證實 myxobacteria 的無限潛力提生產新的自然產物。專注於這個方面, 將會提供 S. cellulosum 基因組上的更新。

（四）Craig A. Townsend

Dr. Townsend 的研究計畫組裡概括有機生物化學裡的天然產物生物合成、次級代謝的酵素及分子生物學。研究有關其物理及合成，廣稱爲生物模仿學合成、酵素物理學、蛋白結構及蛋白工程，探索生物合成酵素的基因結構和 over-expression，研究設計合成油性酸合成抗化劑帶領到實際治療癌症、結核及肥胖症。

β-Lactam 抗生素生物合成的合成物,機械作用和工程學的研究

β-內醯胺機抗生素保持在傳染性疾病的治療。4 組天然發生類別已被發現，現在已被區別在生物化學路徑上。這個會聚性路徑的維持證實了生產有機體的價值。他們也展現 impressive 合成物化學功效，如聰明的改進變主要新陳代謝功能及專門化的生物合成任務。一個轉質會在此詳解這些天然合成產物。

7 月 24 日下午：Poster Session I，共有來自各國共有 144 篇研究論文摘要。

7 月 25 日早上專題演講主題「海洋天然生物資源及技術開發」:

（一）Michael Roberge

Dr. Roberge 研究計畫其中之一項關心到認識 G2 及 M phase 細胞週期的規則及其癌症療法關係。另一項關心到癌症細胞侵略、angiogenesis 及轉移。出版過 70 科學篇章。

以酵母爲對目標藥物遮蔽工具和研究藥物反應的機制

從自然來源被分離出的次要代謝物是有價值的研究工具和引導發現可作爲藥物的化合物。越來越多地, 基礎細胞的顯現化驗來顯示對作用於調節複雜細胞作用的化合物的自然萃取物，像是有絲分裂、 DNA 損害回應或者癌細胞的侵略。不同於在試管內基礎活動力的現顯化驗, 這些顯現用適當的藥物的屬性來確認最初的化學製品, 如同穿過細胞膜的能力。然而, 找到這些化學製品的直接目標也許是一個主要挑戰，因爲他們影響的路徑往往是複雜且又不是十分的理解。我們使用的方法包括：接近的候補選擇, 藥物本質的色層分離法和磷酸化蛋白質研

究。在許多情況需要有系統的和公正的方法去確認他們的作用目標。一個因藥物引起的 haploinsufficiency,，降低基因編碼的劑量的藥物，作用目標由 2 變成 1 時會提高藥物的靈敏性。把這個原理運用在酵母研究 motuporamines 作用機制，marine sponge alkaloids 抑制癌細胞侵略和血管新生，我們使用酵母成長恢復試驗,來結合用特殊蛋白標定的基礎細胞顯現的理想性質。我們使用這個方法確定了抑制免疫的酵素的新的抑制劑 indoleamine 氧化酶, 這應該適用於許多不同蛋白質目標物。這樣, 例如酵母的模型有機體, 理解其基因組容易操控, 能夠在藥物發現方面被利用。

（二）Thomas Murray

Dr. Myrray 是國家藥物濫用協會的藥物濫用生物藥學復審委員會通常委員。把持多個研究計畫：受體信號轉導、神經毒物學及海洋天然產物藥理學。

神經活躍的海洋天然物的發現及機制：敘述大量篩選及神經單位

從神經活躍的海洋天然物標的發現及描述，主要配合大量使用未受傷害的神經單位來做培養。鑑於這些組織擁有著受體及離子管道的重要分子及機能差異，哺乳動物神經元培養的利用優化了化合物偵測的可能性。中樞神經系統與末梢組織比較有正確的表述。合同天然產物化學博士 Dr. William Gerwick，這個入口帶領著我們識別新神經活躍分子。Antillatoxin(ATX)，一個脂肪縮氨酸來源於熱帶海洋藍綠菌 lyngya majuscala。Antillatoxin 是個神經毒性，主要在鼠小腦細粒神經元(GGN)培養及啓動一個細胞內 Ca2+ 的快速增長在這些細胞中。細胞內的 Ca2+的快速增長是以鈉管道對抗藥河豚毒素(TTX)對抗。更多 ATX 與鈉管道電壓閘道互動的直接證明是從 ATX-誘發的 22Na+ 匯入和興奮 [3H]箭毒蛙鹼結合未受傷害神經元來論證。Kalkitoxin 也是來自 lyngbya majuscala 及其比照 ATX，扮演電壓閘道鈉管道的 functional antagonist。這個探討，單分子目標產出催化及抗化劑已有前例。現在這個發現及標示這些新神經活性天然物，提供新藥理學工具來探討中樞神經系統的主要信號影響的機能作用。

（三）Junichi Tanaka

J T 在 Ryukyus, Okinawa 大學學海洋科學。2002-2003 是 Wisconsi-Madison 大學的來訪研究員。他的興趣是從昏暗地帶珊瑚礁發現在藥物及在海洋產物的生活實踐科學。

肌動蛋白目標 macrocycles 及從海洋生物轉化棲息者類固醇

一個龐大的數目細胞毒素分子已被報導，但尚無這些海洋生物的詳細動作機制。Trisoxazole 例如 halichondramide 及 swinholide A 是有特色的海洋 macrocycles。本篇主要在探討它們的肌動蛋白動作。它們的立體結構、黏合處、capping 及其他機動蛋白的行爲。另報告有關 hippuristanols 的抑制、Isis hippuris 的 polyoxygenated 類

固醇。

7 月 25 日下午其專題演講主題爲「天然藥物的二次代謝產物開發」：

（一）Rodney Croteau

Dr. Croteau 在馬省大學取得他的學士及博士學位，他在奧勒剛州立大學花了兩年時間做後博士研究爾後在華盛頓州立大學接受教員(1972 年)。他是植物 terpenoid 化學及生物化學領域的主要專家，taxol 及 monoterpenes 的生合成上有所貢獻。發行超過 350 化學、生物化學、植物生理學及分子遺傳學教材、書籍、書刊、期刊。

Taxol 生合成及遺傳分子學

抗癌症藥物水松(紫杉)種的泰克索生物合成牽涉 19 個步驟，一般的 diterpenoid 前驅物 geranyl-geranyl 雙磷酸鹽取得於 plastidial 甲基丁四醇磷酸鹽；isoprenoid 前導供應途徑。接著經分類而成的同骨骼 committed cyclization，8 細胞色素 P450-調控氧化，3 CoA-dependent acyl/aroyl 轉換，一個 C9 氧化，及 oxetane (D-環) 構成產生中間 Baccatin III，是重要的功能 C13-側鏈連結於 5 個額外步驟。爲取得 taxol 生合成更多內容及其相關能夠改善生產，及描寫推斷其架構、規則及起源，希望大量取得其二次代謝物，以提供市場上的需求。也希望能從遺傳學工程來達成更有效率生物合成量產 taxol 和其前驅物。

（二）Jonathan e. Page

Jon Page 的榮譽論文是藥物性植物的化學根據使用於黑猩猩。在此大學 G.H. Neil Towers 教授下取得博 Ph.D. (1998)。博士論文爲紫菀科的 thiarubrines(有毒的 硫化物 多乙炔)的光化學及光生物學。他的研究任務包括研究乙醯輔酶 A 綜合體。。他的進行中研究著重在分子剖析大麻科(hops and cannabis)的 Terpenophenolic 生物合成及功能性基因表現。

在大麻科中的 Terpenophenolics 的生合成：一個啤酒花和大麻的新陳代謝工程的架構

自 Cannabaceae 家族中，從一系列 terpenophenolic 天然物(prenylated polyketides)被找到有趣的生物活性。於 hops、humulus lupulus L.中之苦味酸及 prenylflavonoids 提供苦味給啤酒及擁有癌症 chemopreventative 特性。Cannabis sativa (大麻類)，一個近似啤酒花的類源植物，非常著名因他的成分對心理或精神有顯著影響的 cannabinoid，Δ9-tetrahydrocannabinol(THC)。該研究嘗試理解生物合成的路徑，terpenophenolics 成形。多數 terpenophenolics 在啤酒花及大麻中的腺狀的毛狀體找，利用生物化學的基因方法，推定乙醯輔酶 A 牽扯到 cannabinoid 複雜的合成路徑。在 hops trichome 基因組計畫發現許多 cDNA 牽扯到苦味酸及

prenylflavonoid 生物合成。這些包括乙醯輔酶 A 合成和其他酵素用於裝飾乙醯輔酶 A 核心架構。了解 terpenophenolic 生合成在分子階層的重要性。

（三）Toni Kutchan

Toni M. Kutchan 教授實驗室的研究主題爲天然植物產物的分子基因學的生合成。他主要的興趣是分離酵素基因密碼及管控蛋白的形成和生理學方面主活動；小分子從 L-tyrosine 或 L-tryptophan(生物鹼)及乙醯輔酶 A(polyketides)。

生物鹼生合成在細胞之間置換的角色

選擇 phenylpropanoids 和 monoterpenoids 生物鹼類累計於特定組織的方式。這些天然藥物及其生合成的分配早已成爲教科書知識。在生物鹼方面，特別是化療藥物 indole 類生物鹼 vinblastine 及 narcotic analgesicmorphinan 類生物鹼 morphine。兩者均未於植物細胞培養上成功製造。利用免疫細胞基因生合成方法，鴉片罌粟累積超過 80 個生物鹼從 l-tyrosine 被識別出來。嗎啡累積在鴉片罌粟的乳液已認知兩個世紀了。先前的生物化學圍繞在鴉片罌粟鹼的多個細胞形式，而免疫細胞化學局部化關於個鴉片罌粟生物鹼形成的 5 個酵素的相關討論。

7 月 26 日：Poster Session II，共有來自各國共有 141 篇研究論文摘要。

7 月 26 日專題演講主題爲「天然藥物在合成上的發展（Synthesis in the Development of Natural Products）」：

（一）Arun K. Ghosh

Dr. Ghosh。在 Harvard 大學研究，他的興趣包括；爲生物學上設計及合成分子探子；Peptidomimetic 的設計及合成；non-peptide mimics 模擬的設計；分子模型製造；發展不對稱合成物的方法論；不對稱的催化作用；天然物製品總生合成。當今是 Illinois, Chicago 的化學教授。有無數的出版品和 15 個專利。

生物活性天然物：如何設計新奇的分子探子

最近 FDA 認可 HIV 蛋白酶抑制劑於結合反轉錄酶抑制劑運用於 AIDS 的化學療法上受到注目。這新治療方法改變了對 AIDS HIV (Human Immunodeficiency Virus) 的進展方向。然而大多數新挑戰排除了重要的 peptide-like 特性和蛋白抑制劑外，該研究設計合成了 non-peptide 蛋白酶抑制劑，成功完成該項計畫大致上可延遲 HIV 攻擊同時緩和 peptide-like。這篇報告主要在介紹天然物的結構、有趣的生物特性及綜合分子探子設計於 HIV 蛋白上的應用。

（二）Michael S. VanNieuwenhze

VanNieuwenhze 教授指導一個第一使用化學合成調製 li pit I 及 lipit II（細菌細胞壁

生合成後階段的中級利用）。她的研究主要著重於搜尋作用劑的合成及機轉族群狀態，研究抑制細菌細胞壁後階生合成路徑。

Peptide 抗生素在抑制微生物細胞壁的生合成

病原體所產生的對普遍所用的抗生素所產生的抗藥性，使之急需能夠抵抗已有抗藥性的微生物的新興抗生素。本篇報告有關 katonosin B(1) 和 plusbacin A3(2) 和 depsipeptide 抗生素；depsipeptide 能夠中斷細胞壁的生合成。

7 月 27 日專題討論：

（一）從南極被囊生物 Synoicum Adareanum 發現一系列新巨環結構得細胞毒成分 Palmerolides A,C,D and E

Synoicum adareanum，在接近南極 Anvers 島收集到一系列被囊生物，詳細描述這一系列新的 polyketide macrolides palmerolide A,C,D 及 E，通常在海綿生物或微生物叢具有巨環特徵。Palmerolide A；在國家癌症研究所(NCI)，60 個人類癌症細胞列模板，相對於其他細胞的測試，其主要顯示有意義有選擇性的試管內細胞毒素來對抗黑色素瘤。Palmerolide C 和 E，兩個結構類似的 Palmerolide A，爲較弱的細胞毒素及對抗黑色素瘤細胞具較少選擇性。基於 NIC 對照分析，Palmerolide A 被發現爲 vATPase 拮抗劑及顯示出結合 V0 副屬單位 與 4 nM 結抗。這篇報告主要在表示 Palmerolides A, C, D and E 的隔離、結構說明、及微生物的活動。

（二）從紅海軟珊瑚 sarcophyton glaucum 取得最佳的生物活性 cembranoid

從海洋來源的 Cembranoids 因它們廣大高產量的生物活動力。Sarcophine；一個主要從紅海軟珊瑚 Sarchophyton glaucum，眾所知的癌症化療藥具潛力的 cembranoid。但是，被報告指出的沒有足夠訊息描述 SAR 研究或這些來自軟珊瑚的其它 cembranoid 的化學預防癌症潛能。基於化學預防及抗炎症性相互關係路徑，我們假設 sarcohpine-有關的 cembranoids 是非常棒的抗癌症及抗炎症作用。Sarcohpines 及 2-epi-16-deoxysarcophine 的分離作爲半合成及生物催化劑的修改 15 個新 cembranoid 衍生物。Cembranoids 的 SAR 的研究試驗他們的效應在高 maignant + SA 乳腺上皮細胞增生和釋放炎症性中介 thromboxane B2 及過氧化物陰離子。有趣的是，硫包含衍生的 sarcohpine ，顯示兩者生物活性增強。其中一個 2-epi-16-deoxysarcohpine 生物代謝衍生物是 14β氫氧基的相似物。這個作用主要藥效基團在預防 cembranoid sarcophytol A and 14β羥基化反應；尚難以合成來達到目的。綜合上述，cembranoids 是個有潛力的抗癌抗炎進程。

（三）自天然產物取得 HIF-1 拮抗劑用於抗乳癌

轉錄因子缺氧誘發因子 (HIF-1)促進腫瘤細胞適應及生存在缺氧環境下。幾千個植物萃取和海洋生物使用拮抗劑 HIF-1 活化的細胞根基的通訊在 T47D 人體乳房

腫瘤細胞作評估試驗。脂質萃取熱帶海洋紅藻 Laurencia intricate 和水棲植物 saururus cernuus 得到在結構上新的雙松烯 laurenditerpenol(IC50 of 400 nM)及奇特的雙新木脂素類稱爲 manassantins(Manassantin B IC50 of 3 nM)。兩系列的化合物在缺氧誘導下，都抑制了 T47D 細胞的 VEGF 蛋白的脈管基因生長。超過 40 個自然發生木脂素類及其他 phenolic-based 類的天然物已被分離及評估。研究結果指出，phenolic-based 類的 HIF-1 抗化劑，許多結構及立體化學特徵強是有力的 HIF-1 拮抗劑活力所必要的。這個研究建議 phenolic-based 類的 HIF-1 拮抗劑可能經由不同機轉可提供不同程度特性而具發展潛力。

（四）約旦河生物多樣性的研究

位於 RTI 的天然物實驗室在抗癌藥物的發現有個漫長的歷史。近來的努力拓展擴展這個研究計畫以一系列生物檢驗來分析不同來源，以得到藥物及農用上的應用。這個合作的計畫焦點是其研究來源有兩個，植物及 hunter bacteria (http://icbg.rti.org/about_research.cfm)。約旦的植物群很特別，他的 4 個生物地理地區允許相互共存種類，有別於其它地區如歐洲、亞西亞、非洲。再者，因爲在非常有限資源下，在那裡差不多有 2500 種類的植物卻很少有系統性的調查。還有 hunter bacteria 是一個在土壤裡發現的非常新興的生物，當正常的條件下，他們取其他微生物和平共存，但是當土質變硬變乾比如缺水、高溫、高鹽度時他們變成了食物鏈上的高者，變成尋食者，殺害其他微生物，所以叫做 hunter (獵人) 細菌。這個細菌的能耐殺害其他微生物顯然地是涉及到抗微生物藥物的研究及發現。約旦粗糙及特殊的地理及氣候環境，包括沙漠高原，熱帶平地，及死海，在有限的狀況下，廣大區域只有少地方可供成熟的研究用。

（五）使用於天然物的藥物探索的進階分析技術

天然產物化學藥物探索在傳統上是個費時的過程。在現今大藥物公司從萃取、分離、純化、活躍有效份子的識別至分子層次是一個非常有挑戰性的 HTS 程序。紅杉已發展到嚴格的 HPLC 萃取及分離法於純化天然產物色析法部分適合現今的 HTS 篩選平臺。紅杉也克服了在結構分析上的即時量如天然物分子的百萬分之一公克量，一個必須要的滿足 “H” 標準在高輸出的篩選平台。一個現今成功的天然產物探索程序需這些快速分離過程且有效的快速結構分析。這些在傳統上需大規模的篩選。

因 CapNMR® 探子的引入，新的機會讓試品需求降低至百萬分之一公克尺寸。紅杉在不久前有報告它的完整過程及許多 CapNMR 探子作用於有限的試品。爲完整 NMR 資料庫合成物的取得，挑戰 HPLC 在百萬分之一公克下的分析，善用最小 NMR 資料於高解析 MS 資料，紅杉可以加快活性及新合成物的發現。

紅杉的萃取及純化過程會特別指出，展示如何使用這新技術來製造天然產物試品的健全管道，標準化 HTS 規則。資料也將展示質子和 COSY 在 1-10 百萬分之一公克的天然物於 HPLC 分析。

（六）位於澳大利亞大學海洋科學天然物研究

澳大利亞是全球生物多樣性的國家其中之一，而且在海洋生態系統中擁有龐大的海洋天然物資源，

澳大利亞海洋科技大學

採集來自熱帶至南、北極的大小微生物樣品，來研究探討天然物的各種學問，我們有專門的技術及設備能夠在化學、分離、生物活性代謝物的結構鑑定。

現在的焦距放在研發新抗癌、抗傳染、抗真菌藥物上，也包括農化物方面，特定除草劑 、除蟲劑等。

參、心得與建議

此次論文成果發表會可以發現，中國大陸代表團之勢力與積極參與國際相關會議之旺盛企圖心，甚至可以看到被遴選參加口頭報告之研究者都相當年輕，反觀國內，包含本人在內的 8 人，遴選參與口頭報告者 1 人（高雄醫學大學天然藥物研究所張教授芳榮），海報張貼有台灣大學及高雄醫學大學等數篇，可以看出國內仍有其成長的空間。

此次行程係屬於相當學術性之研究論文發表會，論文發表方式除了大會特別演講（Plenary Lectures）及邀請演講（Invite Lectures）外，另有口頭報告論文及壁報論文。來自各國之學者無不藉此機會，互相觀摩也互通有無，蒐集資料及認識各個領域之專家，以便未來在研究上得以尋求支援或協助。

報告人此次有幸參加美國生藥學年會，得以增廣見聞，惟由於報告人並非研究領域之專家或學者，尚無法將相關研討內容詳實紀錄，僅能就國外研究方向蒐集相關訊息以作爲制定相關政策之參考。報告人也藉由參加該學術性之會議，反觀目前國內中草藥生物科技推動情形，略以幾個方向提出心得分享：

一、國外中草藥科技研究發展與開發方向：

報告人所參加的年會是一個類似於國內天然藥物研究成果論文發表會，其中發表範圍之廣，涉及以分子模擬輔助藥物設計爲新藥開發之趨勢，新的分子標靶包括酵素、DNA、RNA 等之功能和結構被解析出來，此一領域更可朝結合有機合成與生合成之方式，發展量產之模式，可以將所開發之新天然藥物及其衍生物進行大量之生物活性之篩選，達成新藥開發運用之目的。另還包括微生物基因組開發、天然物活性成分的篩選及藥理作用的探討等，活性成分之篩選不外乎著重於癌症方面之研究與開發，希望繼 Taxol 之後，能自天然物中再成功找出新活性成分順利進入臨床試驗，造福癌症患者。此外，也有利用 Taxol 二次代謝產物來合成大量生產之技術探討。另外，因爲地球四分之三都是海洋，且在陸地上天然物，尤其是植物活性成分或新成分結構幾乎已被篩選殆盡的現在，由此次會議也發現，愈來愈多研究方向朝向海洋資源生物活性成分之篩選並運用於癌症藥物上的研究與開發。

由於天然物篩選活性成分進而開發成爲新藥，須耗費至少十年以上與上億美

金，在如此漫長的過程中，准許經過安全性評估，於人體臨床試驗階段同時，得以膳食補充品方式問世，並於全球佔有很大的市場。

二、國內中草藥生物科技產業推動概況：

近年來美國、德國、日本、韓國、澳洲及中國大陸等國皆開始關注中草藥製藥產業。而世界衛生組織（WHO）也陸續發表相關傳統醫學/另類醫學的文章中，不難發現中草藥正風行於全球；據其資料更顯示全球有八成之人口使用中草藥，故發展中草藥製藥產業將是 21 世紀最具潛力的新興產業。也由於常見疾病、多發病、難治病、甚至一些慢性病尚未有滿意的治療藥物，同時化學藥品長期使用後，發現有各種毒副作用，使得世界各國開始流行的回歸自然，崇尚天然藥物。目前全世界天然藥物市場，每年以 10-20%的速度大幅成長，美國在 1992 年在美國國家衛生研究院（NIH）成立傳統醫學辦公室 OAM，1998 年更擴大爲國立互補替代醫學中心（National Center for Complementary & Alternative Medicine）（NCCAM）1994 年美國國會通過營養補充品（Dietary Supplement）立法，引起全世界對天然爲極大的興趣。2000 年由 FDA 公佈植物性產品的規範“Guidance on Botanical Products“鼓勵各廠家對於植物性產品進行研究，以提供安全、有效的植物性產品。

我國行政院科技技術處認爲 21 世紀要有新的產業推動，在民國 84 年 8 月 30 日行政院通過「加強生物科技產業推動方案」，當時列舉優先發展產業中及包括科學化中藥。86 年 4 月行政院召開首次「生物技術產業策略會議（SRB）」86 年 8 月之「加強生物科技產業推動方案」發展策略中，明定中藥科學化爲重點發展項目之一。以「中藥發展策略」爲主要議題，達成加速中藥研究與篩選，結合學術研究單位及產業界力量針對特定疾病進行研發新藥並嘗試在國內進行臨床試驗，以創造一個成功的模式之共識。87 年第二次 SRB 將中草藥產業列爲國家重大發展目標之一，88 年 5 月第三次 SRB 會議，更進一步闡示如何讓「台灣製造」的品質得到世界認可，包括原料到最終產品。

現階段除了其他相關單位分工於中草藥新藥研發、品管、炮製技術研發等，本署中醫藥委員會在配合國家中藥發展策略的同時，希望能藉由全世界回歸自然風潮且台灣佔有中國幾千年來人類使用中藥的歷史，在安全性較高的的優勢下，中藥新藥臨床試驗可以縮短期程，讓中藥產業再現契機，所負責的「中藥新藥查驗登記須知」及「中藥臨床試驗法規」也已完成就緒。經過幾年的努力，於 2005 年 3 月誕生國內第一件遵循中藥 GCP 規範通過 IND & NDA 之新藥-「紅麴」用於治療高膽固醇血症及高三酸甘油脂血症。代表國內生技產業具有研發新藥（中藥）之能力，更顯示我國中藥之審查品質，已能依照現代化及科學化方式進行，有助於激勵國內業者，進一步依國際審查慣例開發產品，並進軍國際市場，開創中草藥新紀元。嗣後有機會亦應積極的參予該類國際性的學術研究成果發表，甚至可將國內中藥科學化的臨床開發研究經驗，帶到國際場合中，讓先進國家瞭解我們爲中藥現代、化科學化所做的努力與成果。

另在國內推動中草藥生物科技發展的範疇中，就天然物中屬於植物化學活性

成分、新結構的開發部份，國內專門研究天然物之學術單位於民國 75 年成立起，每年舉辦研討會，今年（2005）已邁入第二十年（屆），在天然物的研究開發上，國內透過每年的「天然藥物研討會」，提供國內天然物領域的研究學者們一個相互研究討論的平台與機會，並邀請國外學者專家來演講，並藉此機會讓國內外相關研究學者有所接交流，在天然物活性成分及新研究技術的開發方面，已能與國際接軌。現在已進入後基因體時代，中醫藥相關的研究開發，也慢慢借重該方面的尖端科技，希望由此來得到一些科學性的研究數據作爲佐證，本署中醫藥委員會近幾年也已經積極投入基因體的研究發展計畫，繼續朝中醫藥現代化、科學化的目標邁進。

另外也屬於中草藥生物科技發展推動中的一環的，應繼續開放「可同時提供食品使用之中藥材」部分，國內業者搭上行政院科技技術處對中草藥生物科技產業的推動計畫的這股回歸自然風潮，一直希望本署中醫藥委員會繼續開放「可同時提供食品使用之中藥材」，平心而論，目前本署中醫藥委員會所管理的中藥，均係收載在我國歷代藥書典籍（如：本草綱目，本草拾遺…）中之單、複方藥品，依其醫療經驗爲背景，非「救荒本草、食用本草」類民眾可當作食物使用之品項。縱使已有不少品項早就融入民眾日常養生中，但卻忽略了它的本質還是藥，「藥」即是「毒」。不能因爲使用的頻繁，在沒有科學數據支持其長期大量使用之安全性的情形下，即可輕易開放爲藥食兩用。同時也忽略了開放當作食用，業者對中藥材可能僅依據藥書中之記載，自行組成配方，於未經臨床毒理藥理方面安全性科學數據當作背景之情形下，直接宣稱特定功效，甚至遊走於法規邊緣。另還可能取中藥材爲原料，卻用別於典籍中所記載之調製方式，甚至改以有機溶媒來萃取，其萃取所得的成分可能已不相同，在只有藥材名稱相同製程不同的情況下，衍生出來的所謂的生技產品，在不進行相關安全性試驗的情形下，又怎能得到國際上之認同。

各界積極督促本署中醫藥委員會在沒有安全性背景值，或未與本署食品衛生處等相關管理單位取得共識與配套的情況下，以開放品項來作爲可推動生技產業的重點政策，沒有配套的情況下陸續開放品項，未來市場上的產品，恐會出現以藥材的功效背景行所謂保健食品之宣稱。建議未來在此議題上，應該先彙整相關管理單位之管理規定及意見，回到源頭認清其本質，開放品項同時應擬訂一套長期大量使用安全性無虞爲前提的方案，使生技產業能真正提昇，讓民眾能使用到安全之產品。

肆、致謝

此次有機會代表本署中醫藥委員會參加美國生藥學會第四十六屆年會，要感謝許多的長官及同仁的鼓勵與支持。感謝本會林主任委員宜信給予這個增廣見聞的機會，並於行前給予應對上的指導，還有研究發展組謝組長伯舟提撥該名額且由該組予以經費補助，更要感謝陳組長崇哲在組內業務繁重之際，仍鼓勵珍惜這樣的學習經驗，另有羅主任秘書淑慧、高級研究員育娟及游技正婉如等在行前積

極關心與協助聯繫國內可能參加該年會之學者專家。此行幸有高雄醫學大學天然藥物研究所吳所長永昌與張教授芳榮之同行與協助，使我在這次的行程中不致孤單與無助，順利完成這次任務，報告人從參與本次國際會議中亦學習到不少寶貴的經驗。

伍、參考資料

行政院「中草藥產業技術發展五年計畫」衛生署中醫藥委員會執行總成果報告第 20 屆天然藥物研討會會議資料

陸、附錄

附錄一　美國參加「美國生藥學會第四十六屆年會（American Society of Pharmacognosy 46th Annual Meeting）」內容摘要影印資料

附錄二　會議相關照片

Frontiers in Bioorganic and Natural Products Chemistry

American Society of Pharmacognosy
46th Annual Meeting
Oregon State University

00000001

46th Annual Meeting of the
American Society of Pharmacognosy
July 23-27, 2005

Oregon State University

FRONTIERS IN BIOORGANIC AND NATURAL PRODUCTS CHEMISTRY

Heinz G. Floss

Professor Heinz G. Floss was born and raised in Berlin, Germany. He studied chemistry at the Technical University Berlin and obtained his Ph.D. in chemistry in 1961 and did his "habilitation" in biochemistry in 1966 at the Technical University in Munich. Following a postdoctorate in biochemistry at the University of California, Davis, he joined the faculty of the Department of Medicinal Chemistry and Pharmacognosy at Purdue University in 1966, where he rose through the ranks to the position of Lilly Distinguished Professor and Head of Department. In 1982 he joined the Department of Chemistry at The Ohio State University as Professor of Chemistry and Department Chairman. In 1988 he moved to the University of Washington, Seattle as Professor of Chemistry and Adjunct Professor of Biochemistry, Medicinal Chemistry, and Microbiology. At the end of 2000 he was awarded Emeritus status at the University of Washington, but continues to maintain an active research program. He has trained numerous Ph. D. and postdoctoral students, many of whom hold academic positions in the US, Europe and the Far East. His honors include the NIH Research Career Development Award of the NIH, the Research Achievement Award in Natural Products of the Academy of Pharmaceutical Sciences, the Volwiler Award of the American Association of Colleges of Pharmacy, a Humboldt US Senior Scientist Award, Honoray Doctor of Science degrees from Purdue University and from the University of Bonn, the Research Award of the American Society of Pharmacognosy, the Kitasato Award in Microbial Chemistry, and he is an Honorary Member of the Kitasato Institute, Tokyo.

Professor Floss' research interests are in the biosynthesis of natural products, currently mainly in the chemistry, biochemistry and molecular biology of ansamycin antibiotic formation, and in the stereochemistry and mechanism of enzyme reactions. He has published over 400 original papers, reviews and book chapters.

FROM ERGOT TO ANSAMYCINS - 45 YEARS IN BIOSYNTHESIS **S:1**

Heinz G. Floss
Department of Chemistry, University of Washington, Seattle, WA 98195-1700

Forty five years ago, the author became involved in the field of biosynthesis, quite accidentally, with studies on the formation of ergot alkaloids during his Ph. D. work. This topic occupied him for another two decades, and some of the results and outstanding issues will be briefly highlighted. Extensions of this interest led naturally in two directions, the study of the stereochemistry and mechanism of enzyme reactions and the biosynthesis of antibiotics. Beginning in the 1980s, advances in the molecular biology of Actinomycetes added a new dimension to the latter field. This will be illustrated with some of our current work on the biosynthesis of the ansamycin antibiotics, the rifamycins, antitubercular drugs, and the maytansinoids, potent antitumor agents.

Fumitaka Kudo, Ph.D.

Fumitaka Kudo received his B.S. in Chemistry at Tokyo Institute of Technology (Japan) in 1994. He then completed his Ph.D., studying the biosynthesis of aminoglycoside antibiotics, in the Department of Chemistry at Tokyo Institute of Technology with Professor Katsumi Kakinuma in 1999. Following this, he moved to USA and joined in the laboratory of Professor David E. Cane at Brown University for postdoctoral studies in the biosynthesis of polyketide antibiotics. In 2001 he moved to the Johns Hopkins University to work with Professor Craig A. Townsend for other postdoctoral studies in the biosynthesis of β-lactam. In 2003, he returned to Japan and began his academic carrier as an Assistant Professor at Tokyo Institute of Technology collaborating with Professor Kakinuma, who recently passed away. Even after the loss of his great mentor, he carries on many projects in biosynthesis of natural products including aminoglycoside and polyketide antibiotics.

S:2

RECENT PROGRESS IN BIOSYNTHETIC STUDIES OF 2-DEOXYSTREPTAMINE-CONTAINING AMINOCYCLITOL ANTIBIOTICS

Fumitaka Kudo*
Department of Chemistry, Tokyo Institute of Technology, 2-12-1 O-okayama, Meguro-ku, Tokyo 152-8551, Japan

2-Deoxystreptamine (DOS) containing aminocyclitol antibiotics are clinically important antimicrobial agents including kanamycin, neomycin, *etc.* The carbocyclic structure of DOS is formed from D-glucose-6-phosphate by the key biosynthetic enzyme 2-deoxy-*scyllo*-inosose synthase (DOIS). We have purified DOIS and identified its gene *btrC* from butirosin-producing *Bacillus circulans*. The butirosin biosynthetic gene (*btr*) cluster was subsequently elucidated. Since most of the open reading frames (ORFs) could not been functionally identified by simple homology search, we envisioned that related biosynthetic gene clusters may give significant information as to the crucial biosynthetic genes for the core structure of the antibiotics. Thus, the neomycin biosynthetic gene (*neo*) cluster has been identified from *Streptomyces fradiae*. As a result, most of the *btr* genes were found in the *neo* cluster, which appears to be responsible for the biosynthesis of the core ribostamycin.

Further, functional analysis of the putative ORFs in the *btr* gene cluster was investigated. In addition to the function of BtrC as DOIS, BtrD and BtrS have been successfully characterized as D-glucosamine-1-phosphate/D-glucose-1-phosphate thymidylyltransferase and doubly functional L-glutamine:*scyllo*-inosose aminotransferase, respectively. Functional analysis of other ORFs is also in progress by gene disruption and enzyme assay using the expressed proteins. The details of these studies will be discussed in the meeting.

00000004

Rolf Müller, Ph.D.

Rolf Müller received his Bachelors degree in Pharmacy from the Rheinischen Friedrich-Wilhelms Universität in Bonn, Germany. He remained in Bonn to work on his Ph.D. in Pharmaceutical Biology under the direction of Prof. Dr. E. Leistner. Dr. Müller then joined the Heinz Floss laboratory at the University of Washington where he studied formation of the aminohydroxybenzoic acid starter unit of ansa-macrolides like rifamycin. From 1998 to 2003 he was a group leader at the German Biotechnology Research Center (GBF) in Braunschweig where he led a program investigating the molecular biology of secondary metabolite formation in myxobacteria. He completed his habilitation in 2000 at the Technischen Universität Carolo-Wilhelmina, Braunschweig. In 2003, Dr. Müller relocated his laboratory to the University of Saarbrücken where he is now Professor for Pharmaceutical Biotechnology. His work continues to focus on the molecular and biochemical principles of secondary metabolite formation in myxobacteria and the heterologous expression and modification of myxobacterial natural product biosynthesis pathways.

EXPLOITING BACTERIAL GENOMICS AND NOVEL HETEROLOGOUS EXPRESSION TECHNOLOGY FOR NATURAL PRODUCTS RESEARCH

S:3

Rolf Müller

Pharmaceutical Biotechnology, Saarland University, Im Stadtwald, 66041 Saarbrücken, Germany, (rom@mx.uni-saarland)

Natural products of microbial origin are often biosynthesized by multifunctional megasynthetases whose genetic engineering and heterologous expression offers considerable promise, especially if the natural hosts are genetically difficult to handle, slow growing, unculturable or even unknown. Myxobacteria are a good example for such slow growing and difficult to handle resources of biologically active secondary metabolites. Using genome wide analysis of the genetic potential of *Stigmatella aurantiaca* to produce bioactive substances of polyketide or nonribosomal peptide origin, it was demonstrated that novel secondary metabolites can be found by genetic screening approaches combined with biochemical analyses. Analyses of the genome sequences of *Myxococcus xanthus* and *Sorangium cellulosum* provide further evidence for the immense potential of myxobacteria to produce novel natural products. Focussing on this aspect, an update on the current status of the *S. cellulosum* genome project will be provided.

In addition, a straightforward strategy that combines the power of advanced DNA engineering (recombiogenic cloning) in *Escherichia coli* with the utility of pseudomonads as the heterologous host for the analysis and mutagenesis of known and unknown secondary metabolite pathways is described. The myxochromide S biosynthetic gene cluster from *Stigmatella aurantiaca* was rebuilt and engineered in *E. coli* to contain the elements required for expression in pseudomonads. The successful production in *Pseudomonas putida*, at unprecedented levels, demonstrates the feasibility of a new approach to the analysis and mutagenesis of these important pathways. In parallel, the heterologous expression of this pathway provides direct evidence for an iterative type I polyketide synthase required for the assembly of an unsaturated polyketide chain in myxochromides. Additionally, module skipping is for the first time demonstrated in a nonribosomal peptide synthetase.

00000005

Craig A. Townsend

Dr. Townsend was an undergraduate at Williams College and received his Ph.D. in organic chemistry from Yale in 1974. After holding an International Exchange Postdoctoral Fellowship from the Swiss National Science Foundation at the Eidgenössische Technische Hochschule in Zürich, Dr. Townsend joined the Hopkins faculty in 1976. He has been a Research Fellow of the Alfred P. Sloan Foundation, a Camille and Henry Dreyfus Foundation Teacher-Scholar and was elected Fellow of the AAAS in 2000. He has served the NIH, the ACS, The Office of Technology Assessment, and is an at-large member of the Council of the Gordon Research Conferences. He is currently on the Editorial Advisory Boards of *Chemistry & Biology* and *Bioorganic Chemistry*.

Research programs in Dr. Townsend's group are broadly in the area of bioorganic chemistry with specific interests in natural product biosynthesis, the enzymology and molecular biology of secondary metabolism and molecular medicine. Underlying these studies are interests in reaction mechanism and synthesis, notably biomimetic synthesis, mechanistic enzymology, protein structure and protein engineering, exploration of the genetic organization and over-expression of biosynthetic enzymes, and the study of and the design and synthesis of fatty acid synthase inhibitors leading to practical treatments in cancer, tuberculosis and obesity.

SYNTHETIC, MECHANISTIC AND ENGINEERING STUDIES OF β-LACTAM ANTIBIOTIC BIOSYNTHESIS **S:4**

Craig A. Townsend
Department of Chemistry, The Johns Hopkins University, Baltimore, MD, U.S.A. 21218

The β-lactam antibiotics remain a mainstay in the treatment of infectious diseases. Four naturally-occurring classes are known, each of which is now recognized to be created by a distinct biochemical path. The preservation of these "convergent" pathways attests to their value to a producing organism. They also reveal impressive synthetic chemistry and efficiency, as well as clever evolutionary changes of primary metabolic functions to new, specialized biosynthetic tasks. A selection of transformations will be described that illustrate these aspects of natural product biosynthesis.

TPI 287 A THIRD GENERATION TAXANE

O:1

James D. McChesney, Jan Zygmundt, James. V. Ferrara, John T. Henri
Rodger Lamb, Jonathan E. Foster, Lloyd Garrick, Christian Sumner,
Sylesh Venkataraman, and Herb Brickman

Tapestry Pharmaceuticals, Inc., 4840 Pearl East Circle, #300W, Boulder, CO 80301

The taxane, paclitaxel and docetaxel, are highly active chemotherapeutic agents for the treatment of common malignancies such as cancers of the breast ovary, prostate, and head and neck. However, many of these neoplasms ultimately become resistant to the effects of currently available taxanes and patients relapse. A taxane that could overcome resistance would be a valuable addition to the oncologic therapeutic armamentarium.

In the design of TPI 287, Tapestry Pharmaceuticals leveraged its extensive paclitaxel chemistry experience and understanding of taxane SAR in order to develop this novel taxane. The specific attributes of MDR independence and retention of binding to modified tubulin were goals of the effort since these two mechanisms are thought to contribute to development of taxane resistance in solid tumors.

In preparation, biological evaluation and preclinical development of TPI 287 will be described.

NEOCLERODANE DITERPENES FROM *SALVIA DIVINORUM* AS A NOVEL SCAFFOLD FOR μ OPIOID RECEPTOR LIGANDS

O:2

Thomas E. Prisinzano,[1,*] Wayne W. Harding,[1] Kevin Tidgewell,[1] Matthew Schmidt,[1] Christina M. Dersch,[2] and Richard B. Rothman[2]
[1]Division of Medicinal & Natural Products Chemistry, The University of Iowa, Iowa City, IA 52242 USA and [2]Clinical Psychopharmacology Section, IRP, NIDA, NIH, DHHS, Baltimore, MD 21224 USA

The opium poppy, *Papaver somniferium*, has been used for centuries for the relief of pain and to induce sleep. Among the most important constituents in opium are the alkaloids morphine and codeine. Many of the opiate agonists and antagonists derived from these alkaloids are essential for the effective practice of modern medicine. However, new agents are needed with fewer side effects, such as tolerance and dependence. Recently, the neoclerodane diterpene, salvinorin A, was reported to be a potent and selective opioid receptor agonist *in vitro* and *in vivo*. Salvinorin A is a hallucinogen isolated from the Mexican mint plant *Salvia divinorum* and bears no structural similarity to classical hallucinogens, dissociatives, or opioid receptor ligands. Currently, *S. divinorum* and salvinorin A are gaining popularity as unscheduled hallucinogens available for purchase over the internet. At present, there is little information available as to why this compound is selective for opioid receptors. As part of our program to develop novel opioid receptor ligands, we have begun to investigate the SAR of salvinorin A at opioid receptors. Here, we report the identification of the first neoclerodane that is a μ potent opioid receptor agonist *in vitro* and *in vivo*. In addition, phytochemical analysis of *S. divinorum* has identified salvinicin B, the first naturally occurring neoclerodane with opioid antagonist activity.

SYNTHESIS AND BIOLOGICAL STUDIES OF MYCOTHIOL ANALOGS FROM *MYCOBACTERIUM TUBERCULOSIS*

O:3

Belhu B. Metaferia, and Carole A. Bewley*
National Institute of Diabetes and Digestive and Kidney Diseases/NIH, 9000 Rockville Pike Bldg 8-1A02, Bethesda, Maryland 20892-0820

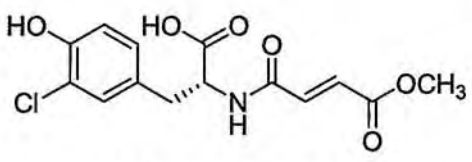
Mycobacterium tuberculosis, the causative agent of tuberculosis, has infected about two billion people worldwide and is a cause for more than two million deaths per year. The demand for potent therapeutic agents has increased due to multi-drug resistance (MDR) and its greater manifestation in patients with HIV/AIDS. One of the plausible strategies in developing new antitubercular drugs is to target the biosynthetic machinery that produces mycothiol (MSH). Mycothiol is a low molecular weight thiol found in actinomycetes, such as *Mycobacterium tuberculosis* with structural features having *N*-acetylcysteine amide linked to GlcN-alpha-(1-1)-*myo*-inositol. Mycothiol plays an important role as a detoxification agent and providing the organism with a reducing cellular environment; hence resistance to drugs and protection from oxidative stress. Previously we have identified a number of natural products from marine invertebrates and terrestrial fungi that inhibit the detoxification enzyme (mycothiol amidase, MCA). As part of our ongoing effort to discover potent inhibitors of mycothiol production, we engaged in the design and synthesis of new substrate mimic analogues that incorporate features of the natural products that appear to contribute to inhibitory activity. Here we report, the synthesis of several mycothiol analogues, and their substrate/inhibitory activities against mycothiol amidase.

CHEMICAL INVESTIGATIONS OF THE FUNGUS *XYLARIA* SP.: NATURAL PRODUCT AND SYNTHETIC STUDIES

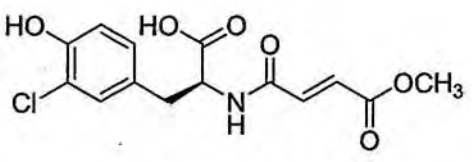
O:4

Rohan A. Davis
Natural Product Discovery, Eskitis Institute, Griffith University, Brisbane, Queensland, Australia 4111

A research program aiming to discover new structural and bioactive metabolites from microfungi isolated from Australian plants has been initiated at Griffith University. Examination of the rainforest tree *Glochidion ferdinandi* has afforded several fungal strains one of which was identified as *Xylaria* sp. (FRR 5657). Chemical investigations of this isolate has recently yielded the new chlorinated natural product (-)-xylariamide A (**1**). Structure confirmation and absolute stereochemistry of **1** were determined by the total synthesis of (+)-xylariamide A (**2**). The synthesis of the natural product (-)-xylariamide A (**1**) has recently been completed using a different synthetic strategy. This paper details the two chemical routes employed in the synthesis of both enantiomers and will report the results from cytotoxicity and antimicrobial screening of **1** and **2**.



(-)-Xylariamide A (**1**)



(+)-Xylariamide A (**2**)

USE OF SIGNAL TRANSDUCTION ENZYME INHIBITION TO TARGET NOVEL BIOACTIVE METABOLITES IN AN ACID MINE WASTE PIT LAKE

O:5

Donald B. Stierle,* Andrea A. Stierle and Kal Kelly, Department of Chemistry, Montana Tech of the University of Montana, Butte, MT 59701

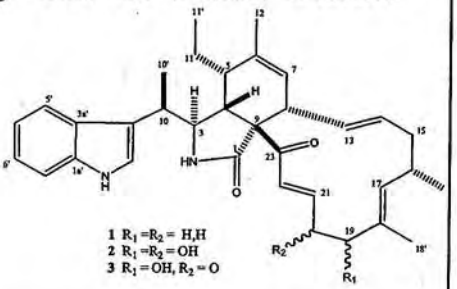
The Berkeley Pit-Lake system in Butte, MT represents a unique biological ecosystem. Its low pH and high metals content give us an opportunity to study microorganisms that have adapted to an extreme environment on this planet. We have isolated more than 40 microorganisms from the waters of this system, from the surface to the sediment layer at 720 feet. Give these organisms a little food and they flourish. We have looked at the secondary metabolites of several of these microbes and found them to be a rich source of new biologically active compounds. We have isolated a unique *Penicillium* sp. that has yielded the unique bicylic ketal, berkelic acid, along with several other metabolites. Berkelic acid showed excellent inhibition against the signal transduction enzyme, caspase-1 and very selective activity against the ovarian cancer cell line, OVCAR-3.

USING JASPLAKINOLIDE TO TURN ON SILENT PATHWAYS THAT ENABLE THE ISOLATION OF NEW METABOLITES FROM FUNGAL CULTURING

O:6

Omar E. Christian,[1] Jennifer Compton,[1] Keisha R. Christian,[1] Susan L. Mooberry,[2] Fredrick A. Valeriote,[3] Phillip Crews.[1,*]
[1]Department of Chemistry and Biochemistry and Institute for Marine Sciences, University of California, Santa Cruz, CA 95064. [2]Southwest Foundation for Biomedical Research, San Antonio, TX, 78245. [3]Division of Hematology and Oncology, Henry Ford Health System, Detroit, MI 48202.

Obtaining new assemblages of natural products from the culture broths of well-studied organisms is not generally possible. We have begun to explore this idea, stimulated by the OSMAC (one strain many compounds) paradigm. Cytoskeletal inhibitors are powerful in their biological action and this present study was begun to explore the possibility that such agents could be used to turn on previously unknown metabolic pathways in marine-derived fungi. The incubation of the marine-derived fungus *Phomopsis asparagi* with the F-actin inhibitor jasplakinolide, resulted in the discovery of three new biologically active chaetoglobosin analogs, chaetoglobosin-510 (**1**), -542 (**2**), -540 (**3**). These compounds were not present in normal culture broths of this organism. All three chaetoglobosins were cytotoxic against various murine carcinoma cell lines and displayed significant actin-disrupting activity. The structures and stereochemical assignments were completed based on spectral analysis.



NEW POLYKETIDE DERIVED METABOLITES FROM THE FRESHWATER FUNGUS *ASCITENDUM AUSTRIACUS*

O:7

Ping Jiao[1], Dale C. Swenson[1], James B. Gloer*[1], Jinx Campbell[2] and Carol A. Shearer[2]
[1] Department of Chemistry, University of Iowa, Iowa City, IA, 52242, and [2] Department of Plant Biology, University of Illinois, Urbana, IL, 61801

Freshwater fungi comprise a distinctive ecological group that has not been widely studied as a potential source of new bioactive natural products. Chemical studies of freshwater fungal isolates in our group have led to the discovery of a variety of new bioactive metabolites, suggesting significant untapped potential among these organisms. During these ongoing studies, several new polyketide metabolites were obtained from an isolate of the newly discovered freshwater fungal species *Ascitendum austriacus*, a member of the family Annulatascaceae. This family occurs commonly on submerged wood in lentic and lotic water habitats. To our knowledge, no chemistry has been reported from this species. The crude EtOAc extract of *A. austriacus* solid-substrate fermentation cultures exhibited modest antifungal activity against *Aspergillus flavus* and was subjected to chemical investigation. These efforts led to the isolation of several new and structurally different types of compounds, including macrolide, benzopyran and benzofuranone metabolites of presumed polyketide origin. Details of the production, isolation, and structure determination of these compounds will be presented.

ASPERGILLAZINES AND ACREMOLIDES: MODIFIED DIPEPTIDES FROM TWO AUSTRALIAN FUNGI.

O:8

Ranjala Ratnayake,[1] Michael Stewart,[1] Ernest Lacey,[2] Shaun Tennant,[2] Jennifer H. Gill[2] and Robert. J. Capon[1]
[1]Centre for Molecular Biodiversity, Institute for Molecular Bioscience, The University of Queensland, St Lucia, Queensland, 4072, Australia, [2]Microbial Screening Technologies Pty. Ltd., Building A, 28-54 Percival Road, Smithfield, New South Wales 2164, Australia

During our ongoing investigations into Australian microbial biodiversity we investigated a series of novel highly modified dipeptides from two fungal species. In this paper we present the isolation and structure elucidation of nine new compounds from two different structure classes, aspergillazines A-E and acremolides A-D, and discuss aspects of their biological properties. Aspergillazine A incorporates a novel fused oxazine/thiophane heterocyclic system while aspergillazines B and C are isomeric analogues in which the oxazine has been reductively cleaved. Aspergillazines D and E are novel tetrahydrofuran analogues of the thiophanes B and C. The structure elucidation of aspergillizines A-E was secured by detailed spectroscopic analysis, which was complicated by the occurrence of both equilibrating epimers and *cis/trans* isomerization about the peptide bond. The aspergillazines possess unique structural features, and offer insights into the reactivity and stability of hitherto unknown heterocyclic systems. The acremolides belong to a family of new lipo-depsipeptides represented by a single reported compound in 2002. All acremolides incorporate a common proline, with variations focusing around a single amino acid residue (Val, Ile, Phe) and the oxidation level of the functionalised fatty acid portion. The structure elucidation of the acremolides was achieved by detailed spectroscopic analysis and chemical derivatization.

Michel Roberge

Dr. Roberge received his B.Sc. and M.Sc. from the University of Sherbrooke and his Ph.D. from the University of Heidelberg. He did post-doctoral training at the University of California at Davis and at the Swiss Institute for Experimental Cancer Research in Lausanne. He joined the faculty at the University of British Columbia in Vancouver in 1991 as an Assistant Professor and he now holds the rank of Professor in the Department of Biochemistry and Molecular Biology. One aspect of his research is concerned with understanding the regulation of the G2 and M phases of the cell cycle and their relevance to cancer therapy. Another aspect concerns cancer cell invasion, angiogenesis and metastasis. His approach is to identify chemicals that affect these processes, use them as tools to study key biochemical components and their interactions, and explore their therapeutic potential. He has published 70 scientific articles and co-edited the book "Cell cycle regulators as targets for cancer therapy". With Raymond Andersen, he received the 2002 Arthur E. Schwarting award for best paper in the Journal of Natural Products for a study describing the discovery of the antitumor agent HTI-286.

YEAST AS A TOOL FOR TARGET-BASED DRUG SCREENING AND FOR STUDYING THE MECHANISM OF ACTION OF DRUGS

S:5

Michel Roberge
Dept Biochemistry & Molecular Biology, University of British Columbia, Vancouver, Canada.

Secondary metabolites isolated from natural sources are valuable research tools and lead compounds for drug discovery. Increasingly, cell-based phenotypic assays are being used to screen natural extracts for compounds that modulate complex cellular functions such as mitosis, the DNA-damage response or cancer cell invasion. Unlike in vitro activity-based screening assays, these screens identify at the outset chemicals with desirable drug attributes, such as ability to cross cellular membranes. However, finding the direct targets of these chemicals may be a major challenge because they affect complex pathways that are often only partly understood. Methods we have taken include candidate approaches, drug affinity chromatography and phosphoprotein profiling. In most cases there remains a need for systematic and unbiased ways to identify their target. One is drug-induced haploinsufficiency, whereby lowering the dose of a gene encoding a drug target from two copies to one often leads to increased sensitivity to the drug. We have used this principle in yeast to study the mechanism of action of motuporamines, marine sponge alkaloids that inhibit cancer cell invasion and angiogenesis. To combine the desirable properties of cell-based screening with targeting of a specific protein we are using yeast growth restoration assays, whereby expression of the target protein interferes with yeast growth while inhibitors of the protein restore growth. We have identified new inhibitors of the immunosuppressive enzyme indoleamine dioxygenase using this approach, which should be applicable to many different protein targets. Thus, model organisms such as yeast, whose genomes are well understood and readily manipulated, can be exploited in drug discovery.

Thomas Murray

Dr. Murray came to the University of Georgia from Oregon State University, where he was Professor of Pharmacology. He spent two years at the National Institute of Mental Health, St. Elizabeth's Hospital in Washington, D.C. as a Pharmacology Research Associate Program fellow from 1979 to 1981. In 1981, he was appointed to the faculty of Washington State University as an Assistant Professor of Pharmacology and in 1983, he joined the faculty of Oregon State University. He is currently a Distinguished Research Professor and Head of the Department of Physiology and Pharmacology in the College of Veterinary Medicine at UGA. Dr. Murray has served as a regular member of the National Institute on Drug Abuse Biomedical review committee and has an active research program in the areas of receptor signal transduction, neurotoxicology and the molecular pharmacology of marine natural products.

DISCOVERY AND MECHANISMS OF NEUROACTIVE MARINE NATURAL PRODUCTS: A TALE OF HIGH THROUGHPUT SCREENING AND NEURONS **S:6**

T.F. Murray, Department of Physiology and Pharmacology, College of Veterinary Medicine, University of Georgia, Athens, GA

Toward the goal of discovery and characterization of novel neuroactive marine natural products, we have utilized intact neurons in primary culture in combination with an array of high throughput screening methodologies. The use of mammalian neuronal cultures optimizes the probability of compound detection inasmuch as this tissue possesses the foremost molecular and functional diversity of receptors and ion channels. Many of these molecular targets are moreover expressed at higher levels in the central nervous system than in peripheral tissues. In collaboration with the natural products chemist Dr. William Gerwick, this approach has led to the discovery and characterization of novel neuroactive compounds. Antillatoxin (ATX), a lipopeptide derived from the pantropical marine cyanobacterium *Lyngbya majuscala.* Antillatoxin is neurotoxic in primary cultures of rat cerebellar granule neurons (GGN) and triggers a rapid increase in intracellular Ca^{2+} in these cells. This rapid increase in intracellular Ca^{2+} is antagonized by the sodium channel antagonist tetrodotoxin (TTX). More direct evidence for an ATX interaction with voltage-gated sodium channels was derived from the demonstration of ATX-induced $^{22}Na^{+}$ influx and stimulation of [^{3}H]batrachotoxin binding in intact neurons. Kalkitoxin is also derived from *Lyngbya majuscala* and, in contrast to ATX, acts as a functional antagonist of voltage-gated sodium channels. The finding that a marine organism produces both an activator and inhibitor of a single molecular target has precedent. The discovery and characterization of these novel neuroactive natural products now provides new pharmacologic tools to probe the functional roles of key signaling effectors in the central nervous system.

Junichi Tanaka

Junichi Tanaka was born in Osaka, Japan and studied Marine Sciences at University of the Ryukyus, Okinawa. He obtained MS from the same university in 1982. He was awarded Ph.D. in Pharmaceutical Sciences from Osaka University under the guidance of Professor Isao Kitagawa in 1990. He was appointed as an instructor of University of the Ryukyus in 1982, and promoted as an associate professor in 1996. From 2002 to 2003, he was a visiting researcher at University of Wisconsin-Madison. His research interests are on drug discovery from coral reef twilight zone and on application of marine natural products for life sciences.

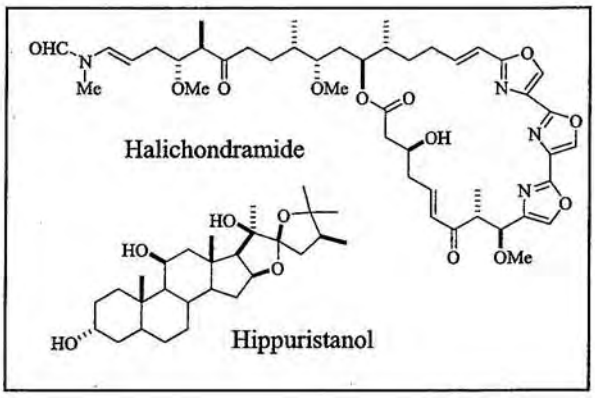
ACTIN-TARGETING MACROCYCLES AND TRANSLATION-INHIBITING STEROIDS FROM MARINE ORGANISMS

S:7

Junichi Tanaka,[1] Chiaki Tanaka,[1] Ayaka Mori,[1] Tatsuo Higa,[1] Vadim A. Klenchin,[2] John S. Allingham,[2] Ryan King,[2] Ivan Rayment,[2] Gerard Marriott,[3] Marie-Eve Bordeleau,[4] Lisa Lindqvist,[4] and Jerry Pelletier[4]

[1]University of the Ryukyus, Nishihara, Okinawa 903-0213, Japan
[2]Department of Biochemistry, University of Wisconsin-Madison, Madison, WI 53706, USA
[3]Department of Physiology, University of Wisconsin-Madison, Madison, WI 53706, USA
[4]McGill University, Montreal, Quebec H3G 1Y6, Canada

A large number of cytotoxic compounds have been reported from marine organisms without detailed mechanisms of action. Trisoxazole macrolides, i.e. halichondramide, and swinholide A are characteristic marine macrocycles. We examined their action on actin. Their stereochemistries, binding sites, capping and other behaviors on actin will be discussed. Also reported here is selective translation inhibition of hippuristanols, polyoxygenated steroids from the gorgonian *Isis hippuris*.



Rodney Croteau

Dr. Croteau received his bachelor's and doctoral degrees from the University of Massachusetts, Amherst, and spent two years on postdoctoral studies at Oregon State University prior to accepting a faculty position at Washington State University in 1972. Rod has remained at Washington State University since that time and now holds the Eisig-Tode Distinguished Professorship in the Institute of Biological Chemistry at Washington State. Rod is a leading expert in the field of plant terpenoid chemistry and biochemistry and has made major contributions in both taxol biosynthesis and the biosynthesis of monoterpenes. He has published more than 350 peer-reviewed articles, book chapters, and reviews in the chemistry, biochemistry, plant physiology, and molecular genetics literature. In addition to his success in maintaining a well-funded research program and consulting for a variety of industries, Rod has had a major impact on the isoprenoid research field by training over 50 M.S. and Ph.D. students and over 75 postdoctoral associates.

TAXOL BIOSYNTHESIS AND MOLECULAR GENETICS

S:8

Rodney Croteau
Institute of Biological Chemistry, Washington State University, Pullman, WA 99164-6340

Biosynthesis of the anticancer drug Taxol in *Taxus* (yew) species involves 19 steps from the universal diterpenoid progenitor geranylgeranyl diphosphate derived by the plastidial methyl erythritol phosphate pathway for isoprenoid precursor supply. Following the committed cyclization to the taxane skeleton, eight cytochrome P450-mediated oxygenations, three CoA-dependent acyl/aroyl transfers, an oxidation at C9, and oxetane (D-ring) formation yield the intermediate baccatin III, to which the functionally important C13-side chain is appended in five additional steps. To gain further insight about Taxol biosynthesis relevant to the improved production of this drug, and to draw inferences about the organization, regulation, and origins of this complex natural product pathway, *Taxus* suspension cells (induced for taxoid biosynthesis by methyl jasmonate) were used for feeding studies, as the foundation for cell-free enzymology, and as the source of transcripts for cDNA library construction and a variety of cloning strategies. This approach has led to the elucidation of early and late pathway segments, the isolation and characterization of over half of the pathway enzymes and their corresponding genes, and the identification of candidate cDNAs for the remaining pathway steps, and it has provided many promising targets for genetically engineering more efficient biosynthetic production of Taxol and its precursors.

Jonathan E. Page

Jon Page was born and raised on Vancouver Island, British Columbia. He studied biology at the University of British Columbia obtaining a B.Sc. (hons.) in 1991. His honors thesis was on the chemical basis of "medicinal" plant use by chimpanzees. He remained at UBC for his Ph.D. (1998), which he completed in the laboratory of Professor G.H. Neil Towers. The subject of his doctoral thesis was the photochemistry and photobiology of thiarubrines, toxic sulfur-containing polyacetylenes from members of the Asteraceae. In 1998 he was awarded an NSERC postdoctoral fellowship and joined the laboratory of Dr. Toni Kutchan in the Institute of Pharmaceutical Biology, University of Munich, Germany to work on molecular aspects of alkaloid biosynthesis. He subsequently moved to the Leibniz Institute of Plant Biochemistry, Halle, where Professor Kutchan was appointed department head, and established a research group in alkaloid functional genomics. His research program also included studies on polyketide synthases from plants. Dr. Page returned to Canada in 2003 to set up a new lab in plant natural product biochemistry at the NRC Plant Biotechnology Institute in Saskatoon. His current research focuses on the molecular dissection of terpenophenolic biosynthesis in Cannabaceae (hops and Cannabis) and functional genomic approaches to alkaloid metabolism.

S:9

BIOSYNTHESIS OF TERPENOPHENOLICS IN THE CANNABACEAE: A FRAMEWORK FOR METABOLIC ENGINEERING OF HOPS AND CANNABIS

Jonathan E. Page
Plant Biotechnology Institute, National Research Council of Canada, 110 Gymnasium Place, Saskatoon, SK, Canada S7N 0W9

Members of the Cannabaceae synthesize a range of terpenophenolic natural products (prenylated polyketides) with interesting biological activities. Bitter acids and prenylflavonoids found in hops, *Humulus lupulus* L., contribute bitter flavour to beer and possess cancer chemopreventative properties, respectively. *Cannabis sativa* L. (marijuana, hemp), a close relative of hops, is well known for its content of the psychoactive cannabinoid, Δ^9-tetrahydrocannabinol (THC). Our research is attempting to understand the biosynthetic pathways by which these terpenophenolics are formed. Based on the fact that most of terpenophenolics found in hops and cannabis are synthesized in glandular trichomes, we are employing a biochemical genomics approach that uses EST sequences derived from these secretory cells. EST sequencing of a cannabis trichome cDNA library has yielded candidates for putative polyketide synthases involved in the cannabinoid pathway, but so far enzymatic activity has been elusive. Our hops trichome genomics project has led to the discovery of several cDNAs involved in bitter acid and prenylflavonoid biosynthesis. These include polyketide synthases and other enzymes that may function to decorate the polyketide core structure. Understanding terpenophenolic biosynthesis at the molecular level may allow the metabolic engineering of this important, and valuable, branch of secondary metabolism in plants.

Toni Kutchan

Professor Toni M. Kutchan studied chemistry at Illinois Institute of Technology (B.S. 1978) and received a Ph.D. degree in Biochemistry from St. Louis University in 1985. After her postdoctoral training at Washington State University in 1986, she joined Professor Zenk's laboratory in Munich, Germany, to work on the biosynthesis and molecular biology of alkaloids. She was habilitated in 1996 and promoted to lecturer in 1997. In 1999, Toni Kutchan accepted the Chair of the Department of Plant Biotechnology at the Leibniz Institute of Plant Biochemistry in Halle/Saale, Germany, and became Managing Director of the Leibniz Institute in 2004. She is affiliated with the Martin-Luther-University Halle-Wittenberg as a Professor of Biochemistry and Biotechnology. A central research theme in Professor Kutchan's laboratory is the biosynthesis of plant natural products at the molecular genetic level. Her main interest is the isolation of genes encoding enzymes and regulatory proteins involved in the formation of physiologically active, small molecules derived from L-tyrosine or L-tryptophan (alkaloids) and acetyl Coenzyme A (polyketides).

A ROLE FOR INTERCELLULAR TRANSLOCATION IN ALKALOID BIOSYNTHESIS

S:10

Toni M. Kutchan
Leibniz-Institut für Pflanzenbiochemie, Weinberg 3, 06120 Halle / Saale, Germany

As for phenylpropanoids and monoterpenoids, selected classes of alkaloids accumulate in a tissue-specific manner. This distribution of many of these classes of natural products and their biosynthesis has become textbook knowledge. In the alkaloid field, special attention has been paid to two pharmaceutically important alkaloids, the chemotherapeutic dimeric monoterpenoid indole alkaloid vinblastine and the narcotic analgesic morphinan alkaloid morphine. Neither of these alkaloids has been successfully produced in plant cell culture, which suggested that cellular differentiation was essential to either their synthesis or accumulation. Given a selection of genes from early and late stages (occurring either before or after branch points in the pathways) of the biosynthesis of vinblastine in the madagascar perinwinkle *catharanthus roseus* and morphine in the opium poppy *papaver somniferum*, the tissue-specific localization of the enzymes has now been investigated with immunocytochemical methods. Multiple cell types are also implicated in the biosynthesis of both classes of alkaloids. Opium poppy accumulates more than eighty alkaloids derived from l-tyrosine, for which many biosynthetic enzymes and an increasing number of genes have been identified. It has been known for almost two centuries that morphine accumulates in the latex of opium poppy. Previous biochemical data suggested involvement of multiple cell types in alkaloid biosynthesis in poppy. The immunocytochemical localization of five enzymes of alkaloid formation in opium poppy will be reported and discussed within context of the results found for monoterpenoid indole alkaloids from *c. Roseus.*

Arun K. Ghosh

Dr. Ghosh received his B.S. with honors in the chemistry at the University of Calcutta in Calcutta, India. He then received his Masters in chemistry and then came to the US to complete his Ph.D. in chemistry at the University of Pittsburgh. He received a Research Fellowship to Harvard University. His research interests include; The design and synthesis of molecular probes for biological systems; Peptidomimetic Design and Synthesis; Design of non-peptide mimics; Molecular modeling; Development of Asymmetric Synthetic methodologies; Asymmetric Catalysis; Total synthesis of biologically important natural products. He is currently a Professor of Chemistry at the University of Illinois at Chicago. He has numerous research publication and 15 patents.

BIOACIVE NATURAL PRODUCTS: HOW DID THEY INSPIRE OUR DESIGN OF NOVEL MOLECULAR PROBES ? **S:11**

Arun K. Ghosh
Department of Chemistry, University of Illinois at Chicago, IL 60607.

Recent FDA approval of HIV protease inhibitors in combination with reverse transcriptase inhibitors has marked a new era of AIDS chemotherapy. The new therapies have changed the course of HIV management and the progression of AIDS. However, the major new challenges are now to eliminate substantial 'peptide-like' character and to combat the emergence of resistance to these protease inhibitors. Our research efforts have been devoted to the design and synthesis of nonpeptide protease inhibitors that are potent against mutant strains resistant to the currently approved protease inhibitors. Successful execution of this approach may substantially delay the emergence of resistant clinical HIV strains and at the same time alleviate the problems of 'peptide-like' character. Our protein-ligand X-ray crystal structure based design and synthesis incorporating natural-product-derived templates resulted in a series of exceedingly potent protease inhibitors. This presentation will feature how natural product structures, their intriguing biological properties and synthesis motivated our design of molecular probes for HIV protease site.

Michael S. VanNieuwenhze

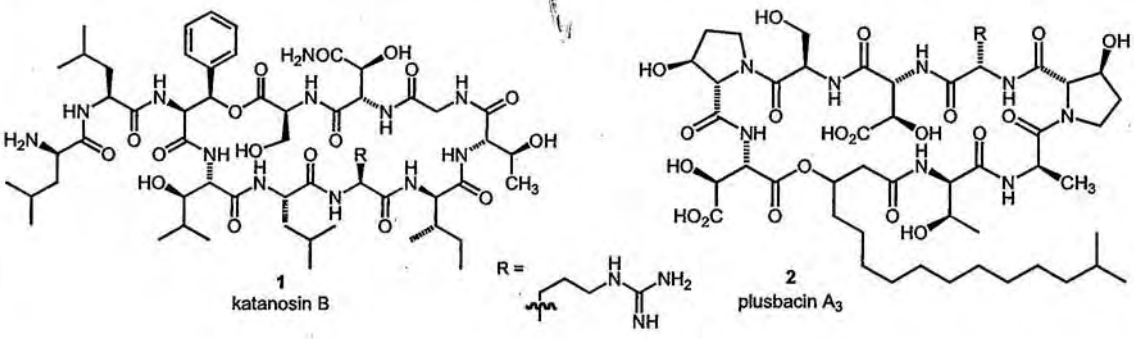
Professor VanNieuwenhze joined the faculty at the University of California at San Diego in 2002 after having spent over eight years in Discovery Chemistry Research at Eli Lilly and Company. While at Lilly, Professor VanNieuwenhze led an effort that was among the first to use chemical synthesis to prepare lipid I and lipid II, late-stage intermediates utilized in bacterial cell wall biosynthesis.

Professor VanNieuwenhze carried his long-standing interest in antibiotics and bacterial cell wall biosynthesis with him to his current position at UCSD. The principal focus of his research group lies in the synthesis and mechanistic study of agents that inhibit the latter-stages of the bacterial cell wall biosynthetic pathway.

SYNTHETIC STUDIES ON PEPTIDE ANTIBIOTICS THAT INHIBIT BACTERIAL CELL WALL BIOSYNTHESIS **S:12**

Michael S. VanNieuwenhze
Department of Chemistry and Biochemistry, University of California, San Diego
9500 Gilman Drive (MC 0358), La Jolla, CA 92093-0358

Bacterial resistance to commonly used antibiotics has created an urgent need for identification of novel antibacterial agents capable of treating infections due to resistant pathogens. Our progress toward the synthesis katanosin B (**1**) and plusbacin A_3 (**2**) and, depsipeptide antibiotics that disrupt bacterial cell wall biosynthesis, will be reported.



1 katanosin B

2 plusbacin A_3

Marc Snapper

Marc L. Snapper received a B.S. in Chemistry/Biology from Union College in Schenectady, N.Y. After four years as a Research Chemist at Sterling Organics (Rensselaer, N.Y.), he entered the graduate program at Stanford University where he obtained a Ph.D. under the guidance of Professor Paul A. Wender. Following postdoctoral studies at Harvard University under the supervision of Professor Stuart L. Schreiber, Snapper joined the Boston College Chemistry Department faculty in 1993 as an Assistant Professor. In 1999 he was promoted to Professor of Chemistry.

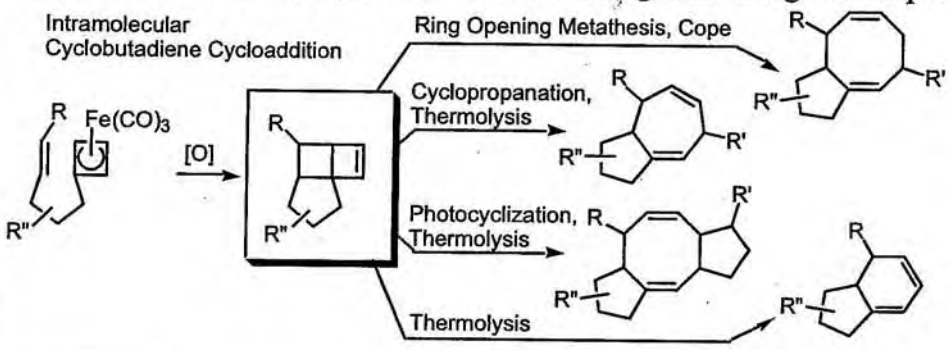
Research interests of the Snapper group include the development of new transformations that allow for the efficient construction of molecules of importance; as well as, the use of these molecules to study biological processes.

CYCLOBUTADIENE CYCLOADDITIONS: NEW OPPORTUNITIES FOR ACCESSING MEDIUM RING-CONTAINING NATURAL PRODUCTS

S:13

Marc L. Snapper
Merkert Chemistry Center, Boston College, Chestnut Hill, MA 02467

New reactions designed at improving access to biologically important organic compounds will be presented. Molecular targets of interest are prepared through highly reactive precursors that lead to strained intermediates, which in turn are rearranged to the desired targets. Specifically, the use of highly functionalized cyclobutenes, prepared through intramolecular cycloadditions of cyclobutadiene, will be highlighted in several new strategies aimed toward the synthesis of medium ring systems. Cyclopropanations, photocycloadditions, and ring opening metathesis of the cyclobutenes will be discussed. The synthetic utility of these transformations will be illustrated with concise syntheses of medium ring-containing natural products.



Frank E. Koehn

Dr. Koehn is Director of Natural Products Discovery and Discovery Analytical Chemistry at Wyeth Research in Pearl River, New York. Dr. Koehn received his Ph.D. degree in Biochemistry from the University of Wisconsin-Madison, in 1983 and went on to a postdoctoral position at the University of Pennsylvania-Monell Chemical Senses Center in Philadelphia. Prior to joining Wyeth in 1994, Dr. Koehn was a senior investigator in marine natural products at Harbor Branch Oceanographic Institution, Ft. Pierce Florida, in the Division of Biomedical Marine Research. Dr. Koehn's research career has been focused on natural products based drug discovery along with development and application of NMR methodology for the characterization of small molecules.

INVERTEBRATE-ASSOCIATED MARINE MICROBES: A RICH SOURCE OF NOVEL CHEMISTRY FOR DRUG DISCOVERY **S:14**

Valerie S. Bernan, Jeffrey E. Janso, Romila D. Charan, Frank E. Koehn, Guy T. Carter
Wyeth Research, Pearl River, NY 10965, USA

Wyeth's marine natural products program increasingly focuses on marine invertebrates as a source for novel actinomycetes and fungi. Taxonomic analysis of the marine isolates has revealed both known genera as well as many unique microorganisms. These cultures have been fermented using a variety of media and evaluated for the production of bioactive compounds in our high-throughput pharmacological screening program. As a result of these efforts new compounds have been isolated and several examples will be presented. In addition to the generation of natural product samples for random screening, we have been attempting to identify and isolate the putative microbial source for the enediyne antibiotics nemenamicin and shishijimicin. These antibiotics are closely related in structure to calicheamicin, which is used in the manufacture of Mylotarg®, Wyeth's monoclonal antibody-directed chemotherapeutic agent for AML. Other than namenamicin and shishijimicin, all previously described enediynes were isolated from cultures of actinomycetes, and it is our hypothesis that a microorganism is involved in the production of these potent cytotoxic compounds. Owing to our continued interest in such compounds as potential 'warheads' suitable for linkage to monoclonal antibodies targeting tumor-specific antigens, microbial isolations were carried out on these ascidians. Using various selective agars, we have isolated numerous actinomycetes, fungi, and eubacteria. The isolates were taxonomically classified using 16S rDNA sequence analysis and Ribotyping™. A combination of detection strategies was employed for production of DNA-damaging activity and biosynthetic capabilities, to prioritize the cultures for further study.

Koty Sharp

Koty Sharp received her B.A. in biology and anthropology from Mount Holyoke College in 1998 and is currently a senior graduate student in Margo Haygood's laboratory at Scripps Institution of Oceanography in San Diego, California. She is interested in animal-bacterial symbioses, and in her thesis research she has focused on bacterial-sponge associations in addition to the symbiosis between the bryozoan *Bugula neritina* and its proteobacterial symbiont, "*Candidatus* Endobugula sertula" to address various aspects of marine bioactive metabolite symbioses. She is especially interested in microbial ecology, host adaptations for transmission and recognition of specific microbes, and species-specificity in sponge-microbial associations. Koty presented her research at the International Symposium for Microbial Ecology (ISME) in 2004 and co-authored a review on molecular techniques in bioactive metabolite symbioses.

BIOACTIVE METABOLITE SYMBIOSIS IN MARINE INVERTEBRATES **S:15**

Koty Sharp
Scripps Institution of Oceanography , University of California, San Diego, LaJolla, California 92093-0202

Many planktonic marine invertebrate larvae, which are conspicous and soft-bodied, utilize bioactive compounds for defense against predators. These compounds play a significant role in shaping the host ecology, and in some cases, the molecules are synthesized by microbial symbionts. The bryozoan *Bugula neritina* harbors a proteobacterial symbiont, "*Candidatus* Endobugula sertula," which has been shown to synthesize the icthyodeterrent bryostatins. We have developed a method for *in situ* bryostatin localization, and our studies demonstrate that bryostatins are most concentrated in the larvae. They also suggest an ontogenetic shift in bryostatin concentration and localization during larval metamorphosis and throughout the host life cycle. The tropical sponge, *Corticium candelabrum*, contains the potent cytotoxic tedanolides, suspected microbial products. Brooding embryos of *C. candelabrum* contain diverse communities of bacteria and archaea. Our investigations of the bacterial communities in *C. candelabrum* identify at least three groups of bacteria that are transmitted via the embryos and are consistently present in *C. candelabrum* across temporal and spatial boundaries. Vertical transmission of the microbes suggests that they play a consistent role in the host ecology, and their role in the production of bioactive compounds for protection of the host larvae is currently being explored. Approaches targeting microbes associated with invertebrate larvae help to focus the search for persistently associated microbes, as well as those that may be the source of chemical protection for larvae.

Christopher L. Schardl

Christopher L. Schardl received a B.S. in Biochemistry at Cornell University in 1978, and a Ph.D. in Biochemistry at the University of California, Davis, in 1983. His postdoctoral research at the Plant Breeding Institute in Cambridge, England, concerned maize mitochondrial genetics. In 1985 Dr. Schardl joined the Department of Plant Pathology at the University of Kentucky, where he now holds the H.E. Wheeler Chair in Plant Mycology, and directs the Advanced Genetic Technologies Center. He is considered the world expert on genetics and evolution of *Epichloe* and *Neotyphodium* species, endophytic fungal symbionts of grasses, which produce several bioactive alkaloids and protect host plants from biotic and abiotic factors. Dr. Schardl's group identified the first gene for ergot alkaloid biosynthesis, identified the precursors of loline alkaloids (1-aminopyrrolizidines) and the loline biosynthesis genes, discovered numerous endophyte species, and demonstrated the importance of interspecific hybridization in endophyte evolution. He now heads the initiative to sequence the genome of *Epichloe festucae*. Dr. Schardl is a fellow of both the American Phytopathological Society and the Mycological Society of America.

BIOPROTECTIVE ALKALOIDS PRODUCED BY EPICHLOË ENDOPHYTES OF GRASSES **S:16**

Christopher L. Schardl
Department of Plant Pathology, University of Kentucky, Lexington, KY, 40546, USA

Many cool-season grasses (fam. Poaceae, subfam. Pooideae) possess endophytic fungi of genus *Eplichloë* or *Neotyphodium* (fam. Clavicipitaceae). Numerous benefits of epichloë endophytes have been documented, particularly protection of host plants from herbivory and abiotic stresses. Four distinct classes of endophyte alkaliods are known – lolines, peramine, ergot alkaloids and indolediterpenes – and many laboratories have studied the pathways, enzymes and genes for their biosysnthesis. All have neurotropic activity against insects, and the ergot alkaloids and indolediterpenes are also active in vertebrates. Much of the ergot alkaloid pathway is known, beginning with synthesis of dimethylallyltryptophan, and proceeding through clavines to lysergic acid and ergopeptines. Recent identification of genes for ergot alkaloid biosynthesis, and techniques to genetically modify fungi enable ecological and physiological roles of these metabolites to be investigated. Likewise, genes have been discovered for biosynthesis of lolines, peramine and indolediterpenes, and endophyte genomics is revealing genes for additional, previously unknown metabolites. These genes can be manipulated for similar studies. Pathways for all four alkaloids involve enzymes encoded in gene clusters, multifunctional enzymes, or both. Expression of the alkaloids in planta is typically much greater than in culture. Thus expression of endophyte alkaloid genes is regulated by communication with the host.

BIOACTIVE METABOLITES FROM OKINAWAN MARINE SPONGES AND TUNICATES

O:9

Haruaki Ishiyama, Masashi Tsuda, Takaaki Kubota, Jun'ichi Kobayashi*
Graduate School of Pharmaceutical Sciences, Hokkaido University, Sapporo 060-0812, Japan

Marine sponges and tunicates have proven to be a good source of compounds with intriguing structures and interesting bioactivities. Recently, several sponges and tunicates were collected at Okinawa, Japan, from which some new bioactive metabolites were isolated and the structures were elucidated by spectral data and chemical means.
Eight dimeric bromopyrrole alkaloids were obtained from a sponge *Agelas* sp., while a dimeric and three monomeric sesquiterpenoids with a nitrogen-containing functionality were separated from a sponge *Halicohondria* sp. A β-carboline alkaloid with an imidazole ring and a brominated bis-indole alkaloid were isolated from the other sponges. These metabolites exhibit inhibition of DNA polymerases or Ser/Thr protein phosphatases, and antimicrobial and cytotoxic activities.
On the other hand, stereochemistry of potent cytotoxic and antitumor macrolides isolated from tunicates *Eudistoma* sp. and *Cystodytes* sp. was assigned by the spectral data and chemical correlations. Since mean graphs of these macrolides in human tumor panels were different from those of known anticancer drugs, mechanism of the action is under investigation.
In this symposium the structures and activities of these marine natural products will be described.

LATRUNCULINS REVISITED: NEW SEMISYNTHETIC BIOACTIVE LATRUNCULINS

O:10

Ashley Barbo,[1] Swapnali Sawant,[1] Diaa Youssef,[2] and Khalid El Sayed.[1]*
[1]Department of Basic Pharmaceutical Sciences, School of Pharmacy, University of Louisiana at Monroe, Monroe, LA 71209. [2]Department of Pharmacognosy, Faculty of Pharmacy, Suez Canal University, Ismailia 41522, Egypt.

Marine-derived macrolides latrunculins A and B, of the Red Sea sponge *Negombata magnifica*, are the first marine natural products that have been found to effectively and reversibly bind to actin monomers and to disrupt its organization. We hypothesized that latrunculins are structurally related to many antimicrobial and antiangiogenic macrolides. The objectives of this study were: 1) to isolate adequate amount of major latrunculins, especially latrunculin A, B, and any related minor macrolides; 2) to chemically optimize the major latrunculin structures using targeted chemical reactions; and 3) to test the antiinfective/antiangiogenic activity of the resulting compounds.
Isolation of latrunculins was achieved using different chromatographic techniques on silica gel and sephadex. Targeted semisynthetic modifications involved C-15-*O*-alkylation, *N*-hydroxy-methylation, dimerization, and acetylation reactions. We were able to isolate several grams of latrunculins A, B, together with a new latrunculin named latrunculin T, from a recent collection of *N. magnifica*. Several new semisynthetic analogs of latrunculins were also generated. Specifically, 15-*O*-methyl latrunculin B showed a promising anti-angiogenic activity in chick chorioallantoic membrane assay, a technique commonly used to measure *in vivo* angiogenic activity. Latrunculins showed potent antimicrobial activity against *Bacillus* and *Saccharomyces* species. We conclude that latrunculins are potential leads that can be developed as antiinfective and anticancer agents.

TWO NEW CYTOTOXIC ALKALOIDS FROM THE RED ALGA *LOPHOCLADIA* SP.

O:11

Harald Gross,[1] Douglas Goeger,[1] Patrice Hills,[2] Susan L. Mooberry,[2] David L. Ballantine,[3] William H. Gerwick*,[1]

[1] College of Pharmacy, Oregon State University, Corvallis, Oregon 97331

[2] Southwest Foundation for Biomedical Research, San Antonio, Texas 78245-0549

[3] Department of Marine Sciences, University of Puerto Rico, Mayaguez, Puerto Rico 00681

Red algae produce a rich and diverse range of secondary metabolites, including polyhalogenated terpenoids, bromophenols, acetogenins, and indoles. Chemical investigation of the red alga *Lophocladia* sp. collected from the Fiji Islands has led to the isolation of two new alkaloids with a 2,7-napthyridine skeleton. The structures were elucidated by employing spectroscopic techniques (NMR, MS, UV and IR). One of the compounds demonstrated phenotypic effects in lung cancer cells and was found to be cytotoxic towards the breast cancer cell line MDA-MB-435 (IC_{50} = 3.1 μM). Further mechanistic studies were carried out and revealed the alkaloid to be a potent and effective microtubule depolymerizing agent (80-85% microtubule loss at 10μg/mL). Due to their resemblance to known bioactive compounds, both compounds were also submitted to the NIMH Psychoactive Drug Screening Program (PDSP) and are currently being evaluated in several neurochemical receptor panels. To date, reports of red algal alkaloids are rare and mostly based on the indole skeleton. Thus, this is the first report of the occurrence of the unprecedented 2,7-napthyridine skeleton from a red alga.

THE FIRST PEPTIDES TO BE ISOLATED FROM THE MARINE SPONGE GENUS *CORTICIUM*

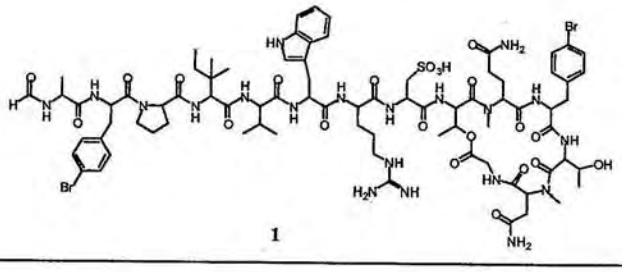
O:12

Damian W. Laird[1], Xidong Feng[2], Tim S. Bugni[1], Mary Kay Harper[1], Guy T. Carter[2] and Chris M. Ireland[1]

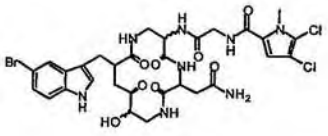
[1] Department of Medicinal Chemistry, University of Utah, Salt Lake City, UT, 84112

[2] Wyeth Research, 401N Middletown Rd, Pearl River, NY, 10965

The sponge genus *Corticium* is widespread in warm and tropical waters of the world's oceans, yet compounds isolated from the genus are far from prevalent within the chemical literature and have limited structural diversity. To date, only a polycyclic alkaloid and a number of steroidal alkaloids have been reported. All reported *Corticium* compounds have exhibited *in vitro* bioactivity. Bioassay-guided fractionation of Fijian specimens has led to the isolation and characterization of two new multi-halogenated cyclic peptides, **1** and **2**, containing a number of unusual amino acids. Structure elucidation was achieved by a combination of extensive NMR experiments and sequencing by HRESIMS with an FTICR instrument.



1



2

CHARACTERIZATION OF BREVENAL: A POLYETHER COMPOUND ISOLATED FROM THE MARINE DINOFLAGELLATE *KARENIA BREVIS*

O:13

Andrea J. Bourdelais[1], Elena Gold[1], Henry Jacocks[1], William M. Abraham[2], Andrew Sayer[3], James E. Gibson[3] and Daniel G. Baden[1]. [1]University of North Carolina-Wilmington Center for Marine Science 5600 Marvin K. Moss Lane, Wilmington NC 28409, [2]Division of Pulmonary and Critical Care Medicine, University of Miami at Mount Sinai Medical Center Miami Beach, FL 33140, [3]Department of Pharmacology and Toxicology, The Brody School of Medicine at East Carolina School of Medicine, Greenville, NC 27834.

A number of bioactive polyether compounds have been isolated from the marine dinoflagellate *Karenia brevis*. The most well-known are a family of neurotoxins called the brevetoxins (PbTx). PbTx bind to site 5 of the voltage sensitive sodium channel in rat brain synaptosomes. A new family of polyether compounds, the brevenals, has been isolated from *K. brevis* and the structures identified using spectroscopic methods. Brevenals consist of 5 fused-ether rings and are effective antagonists to PbTx in all experiments tested including: fish bioassay, comet assay, mucus transport experiments and bronchoconstriction in sheep. Brevenal was also found to displace [^{3}H]PbTx-3 from its binding site in rat brain synaptosomes suggesting that brevenal and PbTx may compete for the same receptor binding site. To test this hypothesis brevenal was reduced to brevenol using [^{3}H]sodium borohydride. The resulting [^{3}H]brevenol was used in a synaptosome binding assay. Cold brevenol and brevenal were able to competitively displace [^{3}H]brevenol but PbTx-2 and 3 did not displace [^{3}H]brevenol at any concentration tested. These results suggest that brevenal and its derivatives may have affinity for another binding site as well as the PbTx binding site.

ISOLATION AND CHARACTERIZATION OF GRIFFITHSIN, A NOVEL ANTI-HIV PROTEIN FROM THE RED ALGA *GRIFFITHSIA* SP.

O:14

Barry R. O'Keefe[1], Toshiyuki Mori[1], Raymond C. Sowder II[2], Scott Bringans[1], Roberta Gardella[3], Shannon Berg[3], Pamela Cochran[3], Jim A. Turpin[4], Robert W. Buckheit Jr.[4], James B. McMahon[1], and Michael R. Boyd[1]
[1]Molecular Targets Development Program, Center for Cancer Research, National Cancer Institute, NCI-Frederick; [2]AIDS Vaccine Program, SAIC-Frederick; [3]Basic Research Program, SAIC-Frederick; [4]Retrovirus Research Laboratory, Southern Research Institute, Frederick, Maryland 21702

Griffithsin (GRFT), a novel potent anti-HIV protein, was isolated from an aqueous extract of the red algae *Griffithsia*. The 121 amino acid sequence of GRFT has been determined by a combination of Edman degradation and enzymatic cleavage. Sequence analysis reveals that it shares no significant homology with previously described proteins or to the transcription products of known nucleotide sequences. We have also produced GRFT recombinantly by expression of a corresponding DNA sequence in *Escherichia coli*. GRFT displayed potent antiviral activity against laboratory strains and primary isolates of HIV-1 with EC50 values ranging from 0.043 to 0.63 nM. In addition, GRFT aborts cell-to-cell fusion. High concentrations (e.g. 783 nM) of GRFT were not lethal to these representative host cell types. GRFT blocks CD4-dependent gp120 binding to the receptor-expressing cells and binds to viral coat glycoproteins but not to sCD4 or other tested proteins. GRFT preferentially inhibits binding of the HIV-neutralizing monoclonal antibody 2G12, which recognizes a carbohydrate-dependent conformational motif and also inhibits the binding of the monoclonal antibody 48d which binds to a CD4-induced epitope.

DOWN-REGULATION OF THE EXPRESSION OF GENES ASSOCIATED WITH INFLAMMATION BY UP446

O:15

Yuan Zhao, Bruce P. Burnett, Qi Jia
Unigen Pharmaceuticals, Inc., 2660 Willamette Drive SE. Olympia, WA 98516

Two polyphenol extracts derived from the roots of *Scutellaria baicalensis* and heartwoods of *Acacia catechu* containing Free-B-Ring flavonoids and flavans, respectively, were combined into a proprietary blend called UP446. In previous studies, we have shown that UP446 possesses cyclooxygenase (COX) and lipoxygenase (LOX) inhibiting activities and exhibits great efficacy in reducing swelling in animal inflammation models. The efficacy was also demonstrated in relieving pain and improving stiffness and function in subjects with rheumatoid arthritis and osteoarthritis in a human clinical trial. In the present study, we discovered that UP446 inhibited not only COX enzyme activity, but also suppressed the gene expression of *cox2* substantially. In addition, UP446 down-regulated the gene expression of a set of pro-inflammatory cytokines, including interleukin-1β (il-1β) and interleukin-6 (il-6). Peripheral blood mononuclear cells (PBMC) from healthy human subjects were used in the experiments. Gene expression of *cox2* and cytokines in PBMC was stimulated with lipopolysaccharide and the effect of UP446 on the gene expression was quantified with realtime quantitative-PCR assays. The results indicate that UP446 may be applied in treatment and prevention of other cytokines mediated chronic inflammation conditions in addition to osteoarthritis.

Referance: Jia, Q et al., The 1st. International Conference on Polyphenols and Health. Opera Vichy, France, November 18-21, 2003.

EFFECTS OF EXT A FROM KOREAN RED GINSENG (*PANAX GINSENG* C. A. MEYER) ON ERECTILE DYSFUNCTION

O:16

Jong Dae Park, Han Jae Shin, Chan Soo Kim*, Won Jae Yang*, Hyung Ki Choi*, Young Deuk Choi*
Div. of Ginseng Research, KT&G Central Research Institute, Taejon 305-805, KOREA & *Dept. of Urology, Yonsei University College of Medicine, Seoul, 120-752, KOREA

Korean ginseng (*Panax ginseng* C. A. Meyer) has been traditionally used for the treatment of various diseases such as cancer, diabetes and cardiovascular diseases as well as improving immune function and stimulating sexual functions. The erectile effects and mechanism of action of the standardized Ext A (total saponin : 62.5 %, PPD saponin/PPT saponin = 1. 45) from red ginseng (steamed and dried ginseng)were evaluated in rabbits and rat corpus cavernosum. On the precontracted rabbit cavernousal muscle strips with phenylephrine, Ext A was shown to increase a significant dose dependent relaxation on control group. After the administration of Ext A, *in vitro* relaxation responses and *in vivo* intracavernousal pressure were increased significantly with a dose dependent and duration dependent manners. After the 3 weeks administration of Ext A at a dose of 100 mg/kg/day, *in vitro* relaxation responses of Ach(acetylcholine) and Ext A were increased significantly compared to control group ($p<0.01$). The intracavernousal pressure to electrostimulation significantly was increased compared to control after the 3 weeks administration of Ext A at a dose of 100mg/kg/day ($p<0.01$). Ext A significantly promoted NOS (Nitric Oxide Synthase) enzyme activity in a dose-dependent manner and also cyclic GMP synthesis, indicating that erectile function of Ext A be mainly mediated by increasing cyclic GMP *via* NO released from the penile corpus cavernosum. These results suggest that Ext A enhance the erectile capacity and clinical trials of Ext A are highly feasible as a therapeutic agent in the treatment of erectile dysfunction.

THE APOPTOSIS-INDUCING EFFECTS OF *CORIOLUS VERSICOLOR* (YUNZHI) EXTRACT IN HUMAN BREAST CANCER CELLS

O:17

Ho CY[1], Kim CF[1], Leung KN[2], Fung KP[2], Tse TF[3], Chan HHL[3] and Lau CBS[1].
[1]School of Pharmacy; [2]Department of Biochemistry, The Chinese University of Hong Kong, Shatin, New Territories, Hong Kong; [3]Vita Green Health Products Company Ltd., Suite 1105, Manning House, 48 Queen's Road Central, Hong Kong.

The fungus, Coriolus versicolor (CV) or Yunzhi (family Polyporaceae), has been shown to exert anti-tumor effects on various cancer cells, but the underlying mechanism has not yet been fully elucidated. The present study aimed to evaluate the in vitro anti-tumor activity of an ethanol-water CV extract on four human breast cancer cell lines using MTT assay, and test whether the mechanism involves apoptosis induction and modulation of p53 and Bcl-2 protein expressions using cell death detection ELISA, p53 and Bcl-2 ELISAs respectively. Our results showed that the CV extract (12.5-800μg/ml) dose-dependently suppressed the proliferation of breast tumor cells T-47D, MCF-7 and MDA-MB-231, while BT-20 cells were not significantly affected. Tumoricidal activity of CV extract was found to be comparable to mitomycin C (anti-cancer drug). Nucleosome productions in apoptotic MDA-MB-231, MCF-7 and T-47D cells were significantly augmented in a time-dependent manner and paralleled the anti-proliferative activity of CV extract. Expression of p53 protein was significantly upregulated only in T-47D cells treated with CV extract in a dose- and time-dependent fashion, but not in MCF-7 and MDA-MB-231 cells. The CV extract significantly induced a dose-dependent down-regulation of Bcl-2 protein expression in MCF-7 and T-47D cells, but not in MDA-MB-231 cells. Our results suggested that apoptosis induction, differentially dependent of p53 and Bcl-2 expressions, might be the possible mechanism of CV extract-mediated cytotoxicity in human breast cancer cells in vitro.

PHTHALIDES AS NUTRACEUTICALS FOR TYPE 2 DIABETES PREVENTION

O:18

Goede Schueler, Claus Kilpert, Laure Morisset, Daniel D'Orazio, Mareike Preller, Antoine de Saizieu, Albine Sorlet, Ying Wang, and Karin Wernli-Kuratli
DSM Nutritional Products, Human Nutrition & Health, Bldg 203/101a, P.O. Box 3255, 4002 Basel, Switzerland

Obesity and type 2 diabetes are becoming epidemic phenomena around the globe. 194 million people between age 20 and 79 were diagnosed with type 2 diabetes in 2003 (International Diabetes Federation 2003). These numbers are expected to increase by 72% between 2003 and 2025.

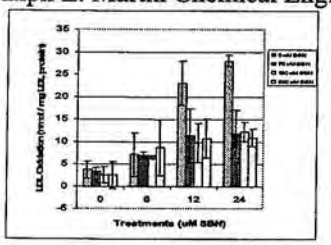
We were looking for natural compounds that could be used as nutraceuticals (e.g. in functional food), and be consumed with the daily diet to increase insulin sensitivity and lower blood glucose level in the long-term and therefore help to prevent type 2 diabetes in people at high risk. In this context the activation of peroxisome proliferator activated receptor-gamma (PPARγ) plays an important role in glucose and lipid metabolism, which is used in pharmaceutical PPARγ ligands (TZDs, glitazones).

We found that phthalides have a strong insulin sensitizing effect *in vitro* and *in vivo*. They show an activity pattern like the synthetic glitazones which we show here for glucose uptake and adipocyte differentiation assays. As examples we present ligustilide, 3-butylphthalide, 3-butylidenephthalide, and sedanolide. They often occur together in various ratios in the plant families *Angelica*, *Ligusticum*, *Cnidium*, and others.

O:19

SILYBININ INHIBITS OXIDATION OF LOW DENSITY LIPOPROTEIN

Danielle Julie Carrier [1], Shanmugam Nagarajan[2], Sunny Wallace[1], Katherine Vaughn[1], and Edgar Clausen[3]
[1]Biological & Agricultural Engineering, 203 Engineering Hall, University of Arkansas, Fayetteville, AR USA 72701
[2] Arkansas Children's Nutrition Center, Microbiology & Immunology, University of Arkansas Medical Sciences, Little Rock, AR USA 72202
[3]Ralph E. Martin Chemical Engineering, 3156 Bell Engineering, University of Arkansas, Fayetteville, AR USA 72701



AIM: Milk thistle (*Silybum marianum* L.), used in the treatment of liver disorders, may also be useful in the treatment of atherosclerosis. **Methods**: In this study the ability of milk thistle flavanolignans silychristin (SCN), silydianin (SDN), and silybinin (SBN) to inhibit chemically-mediated oxidation of low-density lipoprotein (oxLDL) was determined. Native LDL was treated with $CuSO_4$ in the absence or presence of flavanolignans (at 0, 75, 150, and 300 μM). Generation of oxLDL was determined by TBARS, electrophoretic mobility and monocyte adhesion assays. **Main Results**: SBN inhibited the generation of oxLDL at 24h time period (as shown above by TBARS results). Similarly, SCN and SDN showed about 68.7 and 73.5 % reductions in LDL oxidation, respectively. The migration of native LDL incubated with $CuSO_4$ was measured as 20 mm; however, when native LDL was co-incubated with $CuSO_4$ and SBN, this reduced the migration distance to 8 mm. Monocytes adhered (52,625 ± 2,441 cell number) to LDL incubated with $CuSO_4$ in the absence of SBN. However, the monocyte adhesion was reduced to 475 ± 361 cells when LDL and $CuSO_4$ were co-incubated with SBN. These results showed that SBN inhibited the generation of oxLDL and subsequent oxLDL-mediated moncyte adhesion, a primary event in the development of atherosclerosis. **Conclusions:** Milk thistle may offer a protective effect against LDL oxidation.

O:20

THE INTRINSIC HEPATOTOXIC COMPOUNDS IDENTIFIED FROM KAVA ROOTS AND THEIR MOLECULAR MECHANISM

Samuel X. Qiu,[1*] Ji-hua Liu,[2] Ping Zhou,[1] Bo-yang Yu, [2]Michael L. Gross[1]
[1]Chemistry, Box 1134, Washington University, One Brookings Drive, St. Louis, MO 63130, USA;
[2]School of Traditional Chinese Pharmacy, China Pharmaceutical University, Shennong road 1, Nanjing 210038, People's Republic of China.

Kava (*Piper methysticum* G. Forster, Oioeraceae), also known as kava-kava, is an herbal shrub that has been used for centuries in the South Pacific as a social beverage and in traditional ceremonial rituals as a symbol of welcome and respect to visiting guests and dignitaries. Historically, it is also reported to be effective in the treatment of a wide range of medical conditions including asthma, rheumatism, syphilis, gonorrhea, headaches, and insomnia. In the past two decades, kava has been used in the West for treating mild and moderate anxiety, stress, insomnia, restlessness, and muscle fatigue with mild or negligible adverse effects. Kava has been one of the ten best-selling herbal preparations. However, in the past few years, severe side effects, including liver damage that may have resulted in a few cases of mortality, have been reported in both Europe and United States. But the responsible components (if any) and the mechanism remained unknown so far.

By bioassay-guided fractionation, we have identified two novel hepatotoxic compounds from kava for the first time. They can induce severe liver damage as evident from in vitro and in vivo studies. The molecular mechanisms have been dissected as apoptosis-inducing and mediating MAPKs and NF-kB pathways [manuscript has been provisionally accepted for publication in ***Science***].

MOLLAMIDE B AND C: NEW PEPTIDES FROM *DIDEMNUM MOLLE* WITH POTENTIAL ACTIVITY IN CNS DISORDERS AND ALZHEIMERS DISEASE **YS:1**

Marwa Donia and Mark Hamann
University of Mississippi

PALMEROLIDES A, C, D AND E, A SERIES OF NEW CYTOTOXIC MACROLIDES FROM ANTARCTIC TUNICATE *SYNOICUM ADAREANUM* **YS:2**

Thushara K. Diyabalanage, Charles D. Amsler, James B. McClintock[2] and Bill J Baker[2*]
[1]Department of Chemistry, University of South Florida, 4202 East Fowler Avenue SCA400, Tampa FL 33620 and [2]Department of Biological Sciences, University of Alabama at Birmingham, Birmingham, AL 33294

Synoicum adareanum, a colonial tunicate collected near Anvers Island, Antarctica, elaborates a new series of polyketide macrolides, palmerolide A, C, D and E which display characteristic macrolide functionality more commonly found in sponges or microbiota. Palmerolide A, the major member of the group, shows significant selective *in vitro* cytotoxicity against melanoma with three orders of greater sensitivity relative to the other cell lines tested, in National Cancer Institute (NCI) 60 human cancer cell-line panel. Palmerolide C and E, two structural analogues of palmerolide A, were found to be moderately cytotoxic and less selective against melanoma cell lines. Based on NCI's COMPARE analysis, palmerolide A was investigated as a vATPase inhibitor and shown to bind the V0 subunit with 4 nM inhibition. In this paper, we wish to report the isolation, structure elucidation and bioactivity of Palmerolides A, C, D and E.

OPTIMIZATION OF BIOACTIVE CEMBRANOID LEADS FROM THE RED SEA SOFT CORAL *SARCOPHYTON GLAUCUM*

YS:3

Swapnali Sawant,[1] Paul Sylvester,[1] Mitchell Avery,[2] Prashant Desai,[2] Diaa Youssef,[3] Alejandro M. S. Mayer,[4] and Khalid El Sayed.[1] [1]Dept. of Basic Pharmaceutical Sciences, School of Pharmacy, Univ. of Louisiana at Monroe, LA 71209. [2]Dept. of Medicinal Chemistry, School of Pharmacy, The Univ. of Mississippi, MS 38677, [3]Dept. of Pharmacognosy, Suez Canal Univ., Ismailia 41522, Egypt. [4]Dept. of Pharmacology, Chicago College of Osteopathic Medicine, Midwestern Univ., IL 60515.

Cembranoids from marine origin are known for their high yields and wide range of biological activities. Sarcophine, a major cembranoid from the Red Sea soft coral *Sarcophyton glaucum,* is reported for its cancer chemopreventive activity. However, there is not enough information regarding the SAR studies or the chemopreventive potential of other cembranoids from this soft coral. Based on correlation between chemopreventive and anti-inflammatory pathways, we hypothesized that sarcophine-related cembranoids are excellent targets for optimization as anticancer and anti-inflammatory leads. Sarcophine and 2-*epi*-16-deoxysarcophine were isolated in high yields and subjected to semisynthesis and biocatalytic modifications yielding fifteen novel cembranoid derivatives. SAR of cembranoids was studied by testing their effect on highly malignant + SA mammary epithelial cell proliferation and release of the inflammatory mediators thromboxane B_2 and superoxide anion. Interestingly, sulfur containing derivatives of sarcophine showed enhanced activity in both bioassays. One of the 2-*epi*-16-deoxysarcophine biocatalytic derivatives was a 14β hydroxy analog. This functionality is a key pharmacophore in the chemopreventive cembranoid sarcophytol A and 14β hydroxylation was found to be particularly difficult to achieve synthetically. In conclusion, cembranoids are potential anticancer and anti-inflammatory leads.

NATURAL PRODUCT-DERIVED HIF-1 INHIBITORS FOR BREAST CANCER

YS:4

Dale G. Nagle

Department of Pharmacognosy, School of Pharmacy, University of Mississippi, University, MS 38677-1848

The transcription factor hypoxia-inducible factor-1 (HIF-1) promotes tumor cell adaptation and survival under hypoxic conditions. Several thousand extracts of plants and marine organisms were evaluated using a cell-based reporter assay for inhibitors of HIF-1 activation in T47D human breast tumor cells. The lipid extracts of the tropical marine red alga *Laurencia intricata* and the aquatic plant *Saururus cernuus* yielded the structurally novel diterpene laurenditerpenol (IC_{50} of 400 nM) and the unusual dineolignans known as manassantins (Manassantin B IC_{50} of 3 nM), respectively. Both series of compounds inhibited the hypoxic induction of the angiogenic growth factor VEGF protein in T47D cells. A structure-activity relationship study was undertaken to investigate which key structural elements were required for the inhibition of hypoxia-induced HIF-1 activation. More than 40 naturally occurring lignans and other phenolic-based natural products were isolated and evaluated. Results of this study indicate that, for phenolic-based HIF-1 inhibitors, several structural and stereochemical features are essential for potent HIF-1 inhibitory activity. This study suggests that phenolic-based HIF-1 inhibitors should be classified into potency classes that may function through dissimilar mechanisms and provide distinctly different degrees of specificity.

STUDIES ON THE BIODIVERSITY OF JORDAN

YS:5

Nicholas H. Oberlies. Natural Products Laboratory, Research Triangle Institute, P.O. Box 12194, 3040 Cornwallis Rd., Research Triangle Park, NC 27709-2194

The Natural Products Laboratory at RTI has a long history in anticancer drug discovery. Recent efforts have worked to broaden and expand this research base by examining diverse source materials, against a suite of biological assays, for a range of pharmaceutical and agrochemical applications. Perhaps the most well developed example of this research center on our studies in the Hashemite Kingdom of Jordan as part of a planning grant with the International Cooperative Biodiversity Groups (ICBG, insert grant number).

This collaborative program focuses on the investigation of two source materials, plants and hunter bacteria. The flora of Jordan is rather unique, as four biogeographical regions permit the concomitant growth of species that might otherwise only be found in Europe, Asia, or Africa. Moreover, due to limited resources, very few systematic natural product investigations have been carried out on the roughly 2500 species of plants found there. Alternatively, hunter bacteria are relatively newly described organisms of the soil. Under "normal" conditions, they coexist peacefully with other microorganisms. However, when the soil becomes stressed, for example, due to low organic matter, limited water, high salinity, or high temperature, these organisms rise to the top of the food chain, killing other microorganisms for their nutrients. This ability to kill microorganisms has obvious implications for antimicrobial drug discovery. The harsh and unique geographical and climatic conditions in Jordan, including desert highlands, tropical lowlands, and the Dead Sea, to name only a few, present a wide breadth of areas ripe for investigation.

STUDIES ON THE BIODIVERSITY OF JORDAN

YS:5

Nicholas H. Oberlies. Natural Products Laboratory, Research Triangle Institute, P.O. Box 12194, 3040 Cornwallis Rd., Research Triangle Park, NC 27709-2194

The Natural Products Laboratory at RTI has a long history in anticancer drug discovery. Recent efforts have worked to broaden and expand this research base by examining diverse source materials, against a suite of biological assays, for a range of pharmaceutical and agrochemical applications. Perhaps the most well developed example of this research center on our studies in the Hashemite Kingdom of Jordan as part of a planning grant with the International Cooperative Biodiversity Groups (ICBG, insert grant number).

This collaborative program focuses on the investigation of two source materials, plants and hunter bacteria. The flora of Jordan is rather unique, as four biogeographical regions permit the concomitant growth of species that might otherwise only be found in Europe, Asia, or Africa. Moreover, due to limited resources, very few systematic natural product investigations have been carried out on the roughly 2500 species of plants found there. Alternatively, hunter bacteria are relatively newly described organisms of the soil. Under "normal" conditions, they coexist peacefully with other microorganisms. However, when the soil becomes stressed, for example, due to low organic matter, limited water, high salinity, or high temperature, these organisms rise to the top of the food chain, killing other microorganisms for their nutrients. This ability to kill microorganisms has obvious implications for antimicrobial drug discovery. The harsh and unique geographical and climatic conditions in Jordan, including desert highlands, tropical lowlands, and the Dead Sea, to name only a few, present a wide breadth of areas ripe for investigation.

THE POWER OF GENOMIC ANALYSIS IN THE DISCOVERY OF NOVEL SECONDARY METABOLITES

O:21

James McAlpine, Emmanuel Zazopoulos, Dan Sørensen, Mahmood Piraee, Ashraf Ibrahim, Mustapha Aouidate, Chris Farnet
Ecopia BioSciences Inc., 7290 Rue Frederick Banting, Saint Laurent, Québec, H4S 2A1, Canada

Genomic analyses of actinomycetes reveal that a typical strain has the ability to produce 10 to 12 distinct, structurally different secondary metabolites (in addition to congenic analogs), and that this capacity has been the object of only surface scratching. The ATTC 43491 strain of *Amycolatopsis orientalis*, deposited as a vancomycin producer, has the genetic loci for the production of at least 10 secondary metabolites other than vancomycin. We chose to seek two of these; one a short Type I polyketide with unusual starter and terminator groups and the other, a small NRPS product with predictably positioned N-methylation and N-hydroxylation and a less readily positioned, N- formylation. Knowledge of the structure greatly simplifies the detection and isolation procedures and facilitates the production of both NCEs. The investigation of their structure is now more a confirmation than an elucidation. The biosynthetic loci, their expression in appropriate media, and the isolation of each of the metabolites will be described. Their structures are confirmed by classical MS, MS/MS, UV and NMR spectral measurements, with full assignment of carbon and proton chemical shifts, following 1D and COSY, HSQC, HMBC and NOESY, 2D experiments. In the search for novel pharmacophores the ability to predict bioactivity is inversely proportional to the novelty of the structure, and indeed in both of these cases the biological activity represents true discoveries.

THE *pat* PATHWAY TO PATELLAMIDES IN *PROCHLORON DIDEMNI*

O:22

Eric W. Schmidt, J.T. Nelson, David A. Rasko, Sebastian Sudek, Jonathan A. Eisen, Margo G. Haygood, and Jacques Ravel
University of Utah, Scripps Institution of Oceanography, and The Institute for Genomic Research

Prochloron didemni is an obligate cyanobacterial symbiont of the ascidian, *Lissoclinum patella*, which is known to contain patellamides and other bioactive molecules. In the course of the *P. didemni* genome sequencing project, we discovered the first reported complete gene cluster to an invertebrate marine natural product and expressed these *pat* genes in *Escherichia coli* to produce patellamides A and C. The *pat* pathway resembles those responsible for lantibiotic and microcin (ribosomal peptide) biosynthesis in biochemical mechanism, although the individual proteins are less than 10% identical to proteins of known function and have many other unique features. Recently, we have begun to demonstrate biochemical function of individual proteins in the *pat* pathway and have also identified related gene clusters from other *Prochloron* spp. The *pat* pathway will be described, and recent progress in characterization will be discussed.

NOVEL ANALOGUES OF GILVOCARCIN ANTITUMOR DRUGS BY COMBINATORIAL BIOSYNTHESIS

O:23

Tao Liu, Lili Zhu, Madan Kharel, Carsten Fischer, Jürgen Rohr*
Department of Pharmaceutical Sciences, College of Pharmacy, University of Kentucky, 725 Rose Street, Lexington, Kentucky 40536-0082

Gilvocarcin V (GV), the principal product of *Streptomyces griseoflavus* Gö 3592, is the most prominent and most active representative of the distinct family of aryl *C*-glycoside antitumor antibiotics with a coumarin-based aromatic core. This class of anticancer drugs shows excellent antitumor activity and remarkably low toxicity. GV is activated by low doses of visible or near UV light. Although the exact molecular mechanisms responsible for the mode of action of GV are still widely unknown, it was shown recently that GV promotes DNA/histone H3-cross-linking when photoactivated by near-UV light. While the vinyl residue was shown to be responsible for the DNA alkylation, it can be safely suggested that the sugar moiety plays a major role for the interaction with histone H3.

To better understand the DNA/GV/histone H3-complex formation and to generate new GV-derivatives with potentially improved anticancer activity, various novel GV-derivatives were generated using combinatorial biosynthetic methods, i.e. by gene inactivation and/or gene recombination, taking advantage of our recently discovered gene cluster of the GV-biosynthesis. A major focus was GV's sugar moiety, and the first derivatives of GV with altered saccharide moieties were generated. In addition, key steps of the GV-biosynthesis, especially the oxidative rearrangement of an angucyclinone intermediate, were investigated. This paves the way to generate new gilvocarcin-type anticancer drugs through manipulation of other pathways involving angucyclinone intermediates.

BIOSYNTHETIC STUDIES ON THE ANTITUMOR AGENT CETONIACYTONE A

O:24

Xiumei Wu,[1] Patricia M. Flatt,[2] and Taifo Mahmud[1,2,*]
[1]Genetics Program and [2]Department of Pharmaceutical Sciences, Oregon State University, Corvallis, OR 97331, USA

Cetoniacytone A is a new anti-tumor drug isolated from an endosymbiotic *Actinomyces* sp. strain Lu 9419 by Zeeck and co-workers.[1] It contains a unique C_7N aminocyclitol unit in its core structure, which is different from that of the anti-diabetic agent acarbose and the antifungal agent validamycin A. In contrast to most of the secondary metabolites belonging to the C_7N aminocyclitol family,[2] which normally have an alkylated nitrogen at the C-1 position, the nitrogen in cetoniacytone A is acetylated and located at the C-2 position. As part of our studies on the biosynthesis of C_7N aminocyclitol-containing natural products, we have been investigating the biosynthetic pathway to cetoniacytone A in *Actinomyces* sp. strain Lu 9419. Preliminary studies carried out in collaboration with the Zeeck laboratory revealed that cetoniacytone A is derived from 2-*epi*-5-*epi*-valiolone, a common precursor of many C_7N aminocyclitol-containing compounds. To further study the biosynthesis of cetoniacytone A, a genomic library of the producing organism has been established in pOJ446 cosmid vector. Using *valA* and *acbC*, the 2-*epi*-5-*epi*-valiolone cyclase genes from the acarbose and validamycin clusters, respectively, as probe, the biosynthetic gene cluster of cetoniacytone A has been identified. The strategy and current results of this project will be discussed.

1. Schlörke, O.; Krastel, P.; Müller, I.; Usón, I.; Dettner, K.; Zeeck, A. *J. Antibiot.* **2002**, 55, 635–642
2. Mahmud, T. *Nat. Prod. Rep.* **2003**, *20*, 137–166

A SMALL PLANT IN THE BIG TRITERPENE FACTORY - CHARACTERIZATION OF *ARABIDOPSIS* OXIDOSQUALENE CYCLASES

O:25

Quanbo Xiong, William K. Wilson and Seiichi P. T. Matsuda
Dept. Biochem. Cell Biol., Rice University, 6100 Main St., Houston, TX 77005

Oxidosqualene cyclases convert the acyclic oxidosqualene to amazingly diverse cyclic skeletons that are precursors of an enormous number of triterpenes. The *Arabidopsis* genome revealed 13 open reading frames that putatively encode oxidosqualene cyclases. We present the progress on a functional genomics project to characterize these genes. The cDNAs were PCR-amplified and heterologously expressed in yeast. Enzymatic reactions were performed in vivo or in vitro with oxidosqualene as substrate. Lipid fractions were subjected to chromatography and spectroscopic analysis. Seven of the oxidosqualene cyclization products have been identified as new triterpene skeletons. Several enzymes were found to be multifunctional, and one of those generates fifteen compounds ranging from mono, bi, tri, tetra and pentacyclic structures. Although few of the primary cyclization products accumulate to detectable levels in the plant, the heterologously expressed *Arabidopsis* cyclases generate at least 30 different products, or nearly 1/3 of all natural products that are structurally consistent with being oxidosqualene cyclase products. Thus the *Arabidopsis* genome possesses substantially more triterpene biosynthetic capacity than is evident from plant extracts. This report discusses the cloning and characterization of these genes, as well as the structural, mechanistic and phylogenetic insights of these findings.

STEROLS FROM HARMFUL MARINE ALGAE: SYNTHESIS AND METABOLISM

O:26

José-L. Giner,[1] Hui Zhao,[1] Mark S. Dixon,[2] and Gary H. Wikfors[2]
[1]Department of Chemistry, SUNY-ESF, Syracuse, NY 13210.
[2]NOAA, NMFS, 212 Rogers Avenue, Milford, CT 06460.

The harmful algae that produce toxins such as saxitoxin and the brevetoxins also often contain sterols with unusual structures. We recently hypothesized that these sterols serve as chemical defenses by interfering with the nutrition and growth of marine invertebrates. These sterols may be refractory to the normal bioconversion of dietary sterols to cholesterol, and may also interfere with the biosynthesis of steroid hormones by mechanism-based inhibition. To test this, methods for the synthesis of substantial quantities of algal sterols were developed and used to prepare specifically ^{13}C-labeled material. This was incorporated into the diet of juvenile bay scallops (*Argopecten irradiens*) and brine shrimp (*Artemia salina*). Analysis by ^{13}C-NMR spectrometry showed the metabolic fates of the sterols. Addition of a sterol bearing the label in a different position was used as a positive internal control in cases where no bioconversion of the test sterol was detected.

PHAEOCHROMYCINS A-E: NEW ANTI-INFLAMMATORY POLYKETIDES ISOLATED FROM THE SOIL ACTINOMYCETE *STREPTOMYCES PHAEOCHROMOGENES* LL-P018

O:33

Edmund I. Graziani[1], Frank V. Ritacco[1], Valerie S. Bernan[1], and Jean-Baptiste Telliez[2]
[1]Department of Chemical & Screening Sciences, Wyeth Research, 401 N. Middletown Rd., Pearl River, NY, 10965 [2]Department of Inflammation Research, Wyeth Research, 200 Cambridge Park Dr., Cambridge, MA, 02140

Five new polyketide metabolites, phaeochromycins A-E were isolated from an actinomycete designated *Streptomyces phaeochromogenes* LL-P018, cultured from a soil sample collected from a riverbank in Westevenger, Germany. Phaeochromycins A and C were found to be weak inhibitors of MAPKAP kinase-2 (IC_{50} = 39 μM and 130 μM, respectively). The structures of the compounds were determined by spectroscopic analysis, primarily two-dimensional NMR, and revealed that phaeochromycins A, B, C, and E were octaketides, elaborated from a C4 starter unit, related to products of the actinorhodin pathway, namely, mutactin, dehydromutactin, SEK34b, and BSM1. Phaeochromycin D is an unusual partially cyclized degraded octaketide intermediate, and while it is likely that the terminal methyl group (C1) of is derived from C2 of malonate, the mechanism by which this occurs remains obscure.

BIOACTIVE SECONDARY METABOLITES FROM THE MARINE ACTINOMYCETES *SALINISPORA*

O:34

Philip G. Williams, Dong-Chan Oh, Eric Miller, Ratnakar Asolkar, Paul R. Jensen, William Fenical*
Center for Marine Biotechnology and Biomedicine, Scripps Institution of Oceanography, University of California-San Diego La Jolla, California, 92093-0204, USA

We recently reported the cultivation of a new group of obligate marine bacteria that is widely distributed in ocean sediments. To date, in excess of two thousand strains belonging to this new actinomycete genus *Salinispora* have been isolated. Initial screening of this bacterium afforded Salinosporamide A, a potent inhibitor of the 20S subunit of the proteasome. Further screening of these strains using a combined approach of bioassay, LC-MS, and phylogenetic analysis has led to the isolation and identification of several new secondary metabolites. Some notable structural features include a bicyclic ketal, a chlorinated glycoside, and 25-membered ring macrolide. The structure elucidations and biological evaluations of these new metabolites will be described in detail. These results provide a clear indication of the tremendous potential of marine actinomycetes as a source of novel secondary metabolites.

TOWARDS THE EXPLOITATION OF MARINE-DERIVED GLIDING BACTERIA AS A NOVEL SOURCE OF UNIQUE SECONDARY METABOLITES

O:35

Markus Nett[1], Özlem Errol[1], Stefan Kehraus[1], Matthias Köck[2], Ilka M. Molitor[1], Gabriele M. König[1]
[1] Institute for Pharmaceutical Biology, University of Bonn, Nußallee 6, 53115 Bonn, Germany
[2] Alfred Wegener Institute for Polar and Marine Research, Am Handelshafen 12, 27570 Bremerhaven, Germany

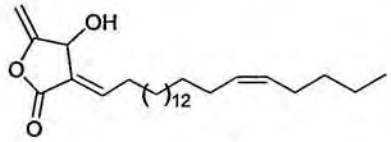
The isolation of unusual microorganisms from habitats that have not been studied so far is a promising approach when searching novel natural products. Myxobacteria have been demonstrated to be an impressive source of structural diversity. Nevertheless reports on natural products from halophilic myxobacteria and other marine gliding bacteria are still scarcely found in the literature.
A collection of 80 sand samples in the intertidal area of the North Sea resulted in the isolation of six gliding bacterial strains showing characteristic morphological features of myxobacteria. Swarms exhibit radial veins of slime and tongue-like extensions at the edge. Older cultures form larger aggregates of slime resembling fruiting bodies of terrestrial myxobacteria. All isolates require a high concentration of salt comparable to that of marine environments for their growth. Phylogenetic analysis based on 16S rDNA similarity of strain TEX2 indicated that the isolate is indeed a myxobacterium and related to *Thaxtera salina*.
Further, the structure of a novel myxobacterial metabolite displaying two oxazole rings with an unusual linkage and a styrene residue will be presented. The structure elucidation which relied considerably on various NMR experiments (amongst others ^{1}H-^{15}N-HMBC and INAPT) and a proposal of a biosynthetic pathway leading to the compound will be discussed.

A NEW CYTOTOXIC HYDROXYBUTANOLIDE ISOLATED FROM THE ROOTS OF A *TAMBOURISSA* SPECIES FROM MADAGASCAR

O:36

Brent Yoder,[1] Shugeng Cao,[1] Andrew Norris,[1] James S. Miller,[2] Fidisoa Ratovoson,[2] Rabodo Andriantsiferana,[3] Vincent E. Rasamison,[3] and David G. I. Kingston[1]
Department of Chemistry, M/C 0212, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061-0212
[1]Department of Chemistry, M/C 0212, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061-0212; [2]Missouri Botanical Garden, P.O. Box 299, St. Louis Missouri 63166-0299; and [3]Centre National d'Application et Recherches Pharmaceutiques, B.P 702, Antananarivo 101, Madagascar

Bioassay-guided fractionation of the root extract of a *Tambourissa* species from the Malagasy rainforest led to the isolation of a new hydroxybutanolide. Techniques used in the fractionation/isolation included liquid/liquid partitioning and normal phase solid phase extraction (both amino and silica columns). Approximately 75 mg of the compound were obtained from slightly more than one gram of starting material. In the A2780 ovarian cancer cell assay, the pure compound demonstrated an IC_{50} of 8 μg/mL. The structure was determined using high-resolution FAB mass spectrometry and optical rotation, as well as ^{1}H, ^{13}C, COSY, HMBC, HSQC and TOCSY NMR techniques.



SOME CHEMISTRY OF A MODERATELY ANTI-FUNGAL EXTRACT FROM *HELICHRYSUM PATULUM*

O:37

V. G. Ntlangwini[1,2], A. Speelman[1,3], Q. Johnson[1,3], B.H. Abegaz[4] and Wilfred T. Mabusela[1,2].
[1]South African Herbal Science and Medicine Institute.
[2]Department of Chemistry and [3]Department of Medical Biosciences, University of the Western Cape, Private Bag X 17, BELLVILLE, 7535, SOUTH AFRICA.
[4]Department of Chemistry, University of Botswana, Gaborone, Botswana.

Fresh aerial parts of *Helichrysum patulum* were extracted with 80% aqueous methanol. The crude extract was shown to have moderate anti-fungal activity. Toxicological studies as performed on Wistar male rats indicated no undue toxicity on the basis of blood analysis as well as histology of the liver and testis.

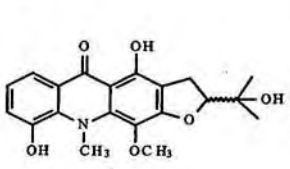
The extract was fractionated by successive solvent partitioning between an aqueous solution and the following organic solvents: hexane, ethyl acetate and *n*-butanol respectively. Since the T.L.C. profiles of these organic extracts were fairly similar, whereas the highest recovery (> 70%) was obtained from the butanol extract, this extract was further fractionated by V.L.C. and column chromatography on silica gel as well as Sephadex LH20. A phenolic glycoside was identified as the major constituent within the polar fractions, whereas the non-polar fractions contained a sesquiterpenoid as the major component, following characterization by n.m.r. and m.s. The broader range of minor constituents was investigated by g.c.-m.s., which indicated the presence of a variety of closely related sesquiterpenoid structures. One of these was also isolated as its glycoside, which was characterized following conversion into its acetylated derivative.

ACRIDONE ALKALOIDS FROM *SWINGLEA GLUTINOSA* (BL.) MERR. AND THEIR ANTIPARASITIC ACTIVITIES

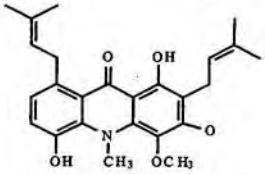
O:38

Djalma A. P. dos Santos,[a] Paulo C. Vieira,[a] M. Fátima das G. F. da Silva,[a] João B. Fernandes,[a] Lauren Rattray [b] and Simon L. Croft [b]
[a]Departamento de Química, Universidade Federal de São Carlos, CP 676, 13565-905 São Carlos - SP, Brazil; [b]Department of Infectious and Tropical Diseases, London School of Hygiene and Tropical Medicine, London, WC1E 7HT, UK

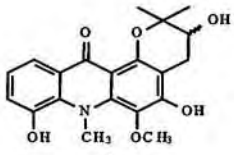
Eleven acridone alkaloids were isolated from *Swinglea glutinosa* (Bl.) Merr., three having novel structures. They have been examined for *in vitro* activity against chloroquine-sensitive *Plasmodium falciparum* 3D7, *Trypanosoma brucei rhodesiense* STIB900 and *Leishmania donovani* L82. An assay with KB cells has been developed in order to compare *in vitro* toxicity of alkaloids with the selective action on the parasites. Nine of the compounds tested had IC_{50} ranging from 0.3 to 11.6 μM against *P. falciparum*. In contrast, a small number of compounds showed significant activity against *T. brucei rhodesiense* and none had activity against *L. donovani*. Among the alkaloids three had $IC_{50} < 1.0$ μM against *P. falciparum*, whereas against *T. b. rhodesiense* five had $IC_{50} < 10$ μM. The characterization of the acridone alkaloids **1**, **2** and **3** will be discussed, as well the structure-activity relationships.



1



2



3

DISCOVERY OF FABH/FABF INHIBITORS FROM NATURAL PRODUCTS USING A NOVEL STRATEGY

O:39

Katherine Young, Hiranthi Jayasuriya, John G. Ondeyka, Kithsiri Herath, Chaowei Zhang, Deborah L. Zink, Andrew Galgoci, Srinivas Kodali, Ronald Painter, Lynn L. Silver, Janet Sigmund, Angela Basilio, Francisca Vicente, Jose Tormo, Olga Genilloud, Fernando Pelaez, Doris Cully, John F. Barrett, Dennis Schmatz, Sheo B. Singh and Jun Wang
Merck Research Laboratories, Rahway, New Jersey 07065

FabH and FabF are essential enzymes in type II fatty acid synthesis and are promising targets for antibacterial drug discovery and development. A new approach using a xylose inducible plasmid to express antisense RNA (AS-RNA) in *Staphylococcus aureus* has been recently described. In order to identify FabF/FabH target specific cell permeable inhibitors from natural products, we developed an agar-diffusion two-plate differential sensitivity assay. Because both the *fabH* and *fabF* genes share the same operon, the increase in *fabF* AS-RNA levels decreases the expression of FabH and FabF proteins, making the cells more sensitive to FabF and/or FabH inhibitors. In this assay, natural product extracts are applied on two plates, one seeded with *S. aureus* cells with expression of *fabF* AS-RNA (AS plate) and the other without expression of AS-RNA (control plate). The extracts that contain selective inhibitors for either of the two targets will form a larger inhibition zone on the AS plate compared to the control plate. The assay was validated with ~80 known antibiotics including fatty acid synthesis inhibitors. Using this assay, we screened over 250,000 natural product extracts followed by confirmation in biochemical assays, giving a hit rate of 0.1%. We discovered all known FabH and FabF inhibitors that included cerulenin, thiolactomycin, thiotetromycin and U-68,204 from natural product extracts for the first time using a mechanism based screening approach. We also discovered new acetylenic allenes as FabF inhibitors providing an opportunity for the discovery and development of novel FabF/FabH inhibitors. The efficacy and selectivity of all purified natural products both *in vitro* and *in vivo* were characterized.

EXPRESSION OF RECOMBINANT MYCOTHIOL LIGASE (MSHC), A NEW TARGET FOR THE SCREENING OF ANTITUBERCULAR AGENTS.

O:40

Maria-Teresa Gutierrez-Lugo, Carole A. Bewley
Laboratory of Bioorganic Chemistry, National Institute of Diabetes and Digestive and Kidney Diseases, National Institues of Health, 9000 Rockville Pike, Bethesda MD 20892.

Mycothiol (MSH) is the major low molecular weight thiol in actinomycetes and is associated with the protection of *Mycobacterium tuberculosis* from toxic oxidants and antibiotics. MSH comprises one unit each of acetylcysteine (AcCys), glucosamine (GlcN) and inositol (Ins). A key enzyme in its biosynthesis is MshC, which transfers cysteine to GlcN-Ins. It has recently been demonstrated that the MshC gene and more generally the production of MSH are essential for the growth of *M. tuberculosis*. Therefore, MshC constitutes a potential target for new drugs directed against *M. tuberculosis*. A biochemical assay was thus developed to identify small molecule inhibitors of MshC. The assay involves the identification of the ligation reaction product, Cys-GlcN-Ins after derivatization with monobromobimane using LCMS. However, the production of the recombinant protein for the bioassay is a major prerequisite. Mycobacterial proteins are difficult to express in heterologous systems due to their poor stability, solubility and toxicity to the host. To overcome this problem, MshC was expressed as GB1, GST and MBP fusion proteins in *E. coli* and *M. smegmatis*. All fusion proteins were well expressed in both hosts. However, despite enhanced expression in *E. coli*, all fusion proteins were mainly produced as inclusion bodies. *M. smegmatis* was found to be a better host for the production of the respective tagged proteins in soluble and active form. Cloning, levels of protein expression, purification, yields, and activity of recombinant MshC are described.

THE TRANSGENIC ARABIDOPSIS PLANT, *pER8-GFP*, AS A POWERFUL TOOL IN SEARCHING NATURAL ESTROGEN-AGONISTS/ANTAGONISTS

O:41

Fang-Rong Chang,[1] Ken-ichiro Hayashi,[2] Nam-Hai Chua,[3] Shuichi Kamio,[2] Zih-You Huang,[1] Hiroshi Nozaki,[2] Yang-Chang Wu*[1]

[1]Graduate Institute of Natural Products, Kaohsiung Medical University, Kaohsiung 807, Taiwan [2]Department of Biochemistry, Faculty of Science, Okayama University of Science, 1-1 Ridai-cho, Okayama 700-0005, Japan [3]Laboratory of Plant Molecular Biology, The Rockefeller University, 1230 York Avenue, New York, NY 10021, USA

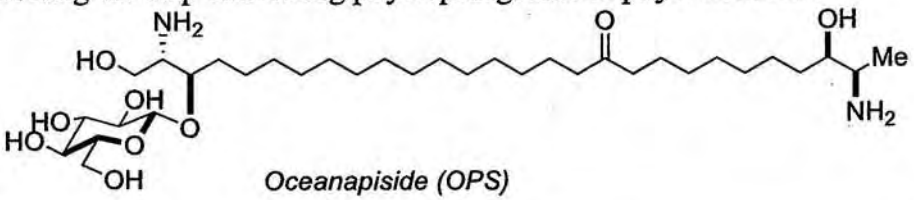
A new and efficient bioassay method has been developed as a powerful tool in searching natural estrogen-agonist/antagonists, by using the transgenic arabidopsis plant, *pER8-GFP*, harboring human estrogen receptor. Four plant methanol extracts as well as 13 pure compounds were assayed based on the rational experimental design. The remarkable outcomes show that this assay could screen estrogen-agonist/antagonists from natural sources. To our understanding, it is the most avant-garde study which first uses the transgenic plant for the estrogenic assay related to animals and human beings.

OCEANAPISIDE, A MARINE NATURAL PRODUCT, TARGETS THE SPHINGOLIPID PATHWAY OF FLUCONAZOLE RESISTANT *CANDIDA GLABRATA*

O:42

Doralyn S. Dalisay, Evan W. Rogers and Tadeusz F. Molinski*
Department of Chemistry, University of California, Davis, CA 95616, USA

Oceanapiside, a marine natural product with a novel bifunctional sphingolipid structure, is *fungicidal* against Fluconazole-resistant *Candida glabrata* at 10 μg/ml. The fungicidal effect was observed at 3 to 4 h after treatment was started. Oceanapiside affects budding patterns of treated yeast cells. Moreover, it inhibits polarized growth and disrupts the organized actin assembly in *C. glabrata*. It was also demonstrated that phytosphingosine reversed the antifungal activity of oceanapiside, suggesting that oceanapiside blocks the pathway downstream of phytosphingosine synthesis. We quantified the amount of long chain bases (LCBs) and phytoceramide from the crude extracts of treated cells using LC-ESI-MS. Phytosphingosine (PHS) level was elevated in oceanapiside treated cells when compared against the crude extracts of cells treated with miconazole and amphotericin B. The elevated levels of PHS in oceanapiside-treated cells confirms that oceanapiside affects the pathway at a step downstream of PHS synthesis. Our study revealed that oceanapiside interdicts fungal sphingolipid metabolism by specifically inhibiting the step converting phytosphingosine to phytoceramide.

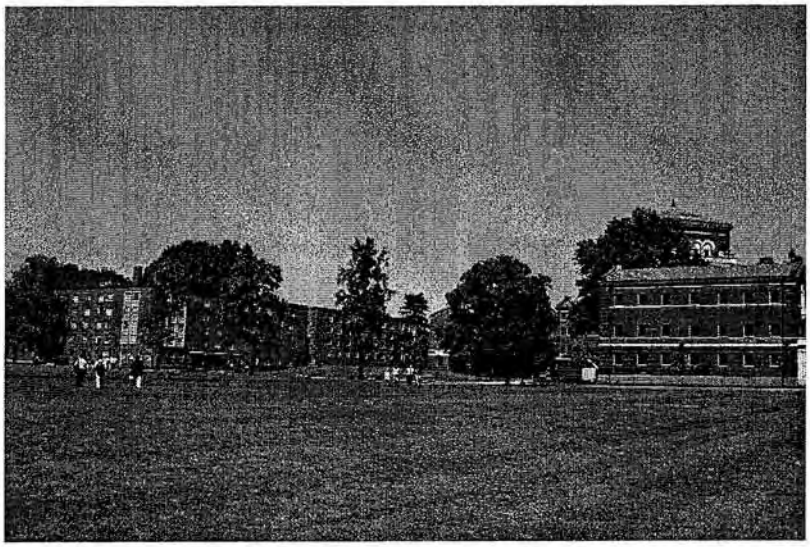


Oceanapiside (OPS)

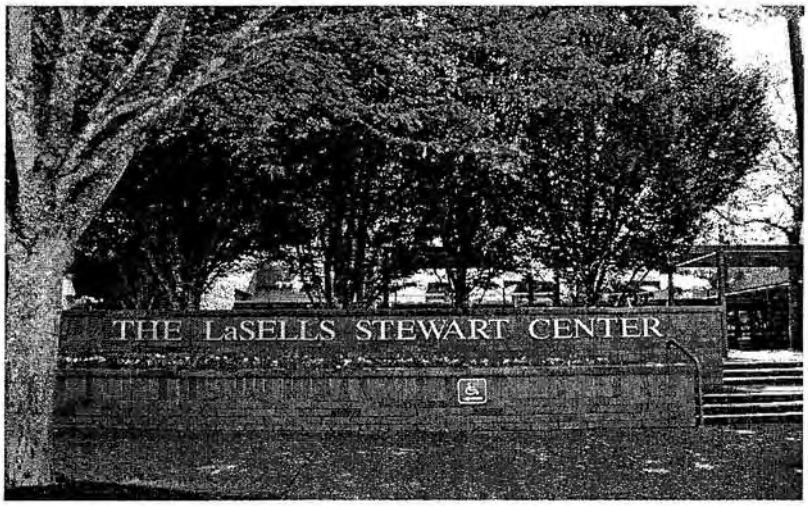
00000040

生藥學年會報到處

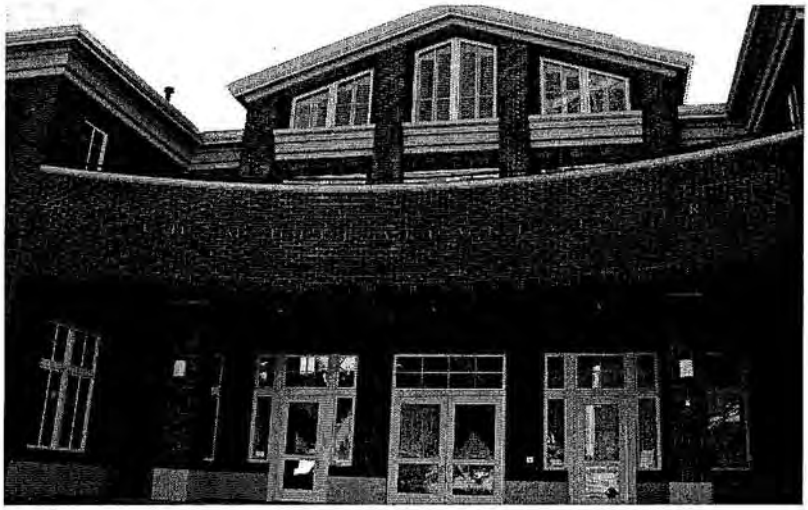
生藥學年會會場外交流



奧勒岡州立大學校園一角



研討會會場之一



研討會會場之二

壁報論文發表