Research Achievement Award—D. John Faulkner†

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David John Faulkner, one of the pioneers of marine natural products chemistry and the 2003 recipient of the ASP Research Achievement Award, passed away on November 23, 2002. John was very pleased to learn that he'd been named as the award recipient and was intending to present the ASP Research Achievement Award Address at the annual meeting in July 2003, Chapel Hill, North Carolina. John's untimely death left an unprecedented event in the history of the ASP—a posthumous Research Achievement Award to a deserving individual and an untimely loss for us all. We are bereft of a colleague, a friend, and a mentor, and the opportunity to hear John's words on the occasion of his own award. In tribute to John, we have assembled a retrospective of John's work that is not meant to be a comprehensive review (this would take considerably more space) but a selection of highlights and personal vignettes from some of those that trained in marine natural products under his mentorship. This paper is a written account of the symposium presented by the authors at the ASP Annual Meeting in Chapel Hill, North Carolina, on July 16, 2003.

"If I have seen further... it is by standing on the shoulders of giants" — Isaac Newton

Introduction

David John Faulkner,[1] who was born and educated in England, developed his career in marine natural products in the United States, and is known to many as one of the pioneers of modern marine natural products chemistry, shared traits with a most famous scientist of an earlier time—Sir Isaac Newton. Both, at heart, were natural philosophers. James Gleick's recent biography[2] of Sir Isaac Newton (1642−1727) portrays the life of Newton as a brilliant if reluctant genius. Gleick's Newton is irascible, difficult, but at other times, inspired, his spirit lifted by those phenomena he sought to understand and the sublime orderliness and precision of the forces that move the heavenly bodies and Earth through the void. He laid order and reason upon fundamental properties of nature—inertia, gravity, optics—and cobbled the tools of calculus, as much out of necessity as of a passionate expression for the underlying mathematics of all things that move and change. The Newtonian revolution rose at the dawn of the age of reason. Yet despite his brilliance, Newton was also taciturn and conflicted by the scientific disagreements with the rivals that he incited. (Robert Hooke and Leibnitz, to name two. Robert Hooke (1635−1703), who is credited with invention of the compound microscope, came to intellectual blows with Newton within the ranks of the Royal Society over the nature of light refraction. On another front, a simmering dispute over the invention of calculus was the source of considerable *angst* between Newton and the German mathematician Gottfried Wilhelm Leibnitz (1646−1716).) Yet, while Newton was unsure of the revolution he had wrought and hesitant to embrace it, Faulkner relished the dawn of the modern age of natural products chemistry. Newton, it has been said, "*was by no means a 'Newtonian'*",[3] but Faulkner was truly 'Faulknerian'.

John Faulkner was trained as a synthetic chemist, but his career was shaped (somewhat reluctantly, at first) by marine science and natural products at Scripps Institution of Oceanography. He shared traits with his fellow Englishman Isaac Newton. They both came from modest economic backgrounds, but elevated themselves through science. In their respective fields, they excelled and made indelible marks on their fields of scholarship. They were at times taciturn and at others passionate of their science; they were iconoclasts in their time.

The First Generation

David John Faulkner joined the faculty at Scripps Institution of Oceanography in 1968 as an Assistant Professor of Marine Chemistry. Having completed a Ph.D. dissertation with Sir Derek H. R. Barton in 1965 and postdoctoral studies with Professors Robert B. Woodward and William S. Johnson, John possessed a strong fundamental understanding of organic and natural products chemistry and was poised to make major contributions to a newly evolving research field that would become known as marine natural products. At that time the discipline was literally in its infancy; nonetheless pioneering work by Scheuer, Hashimoto, Hirata, and others on toxins produced by marine organisms along with the first 'Drugs from the Sea' symposium held in Rhode Island in 1967 clearly illustrated the vast potential of the world's oceans as a source of unique chemistry and chemical diversity. John Faulkner and his first generation of graduate students, from 1971 to 1979, fully capitalized on this burgeoning interest in marine natural products. This first generation yielded seven Ph.D. theses and one Masters thesis. John coauthored more than 50 papers with his students during this period (Table 1 provides a list of John's first students). Many of the papers John's group published during this

† Dedicated to the late Dr. D. John Faulkner (Scripps) and the late Dr. Paul J. Scheuer (Hawaii) for their pioneering work on bioactive marine natural products. Presented at the ASP Research Achievement Award Address, July 16, 2003, Chapel Hill, NC.

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Table 1. The 'First Generation' of Faulkner's Students, Degree Obtained, Graduation Date, and Thesis Title

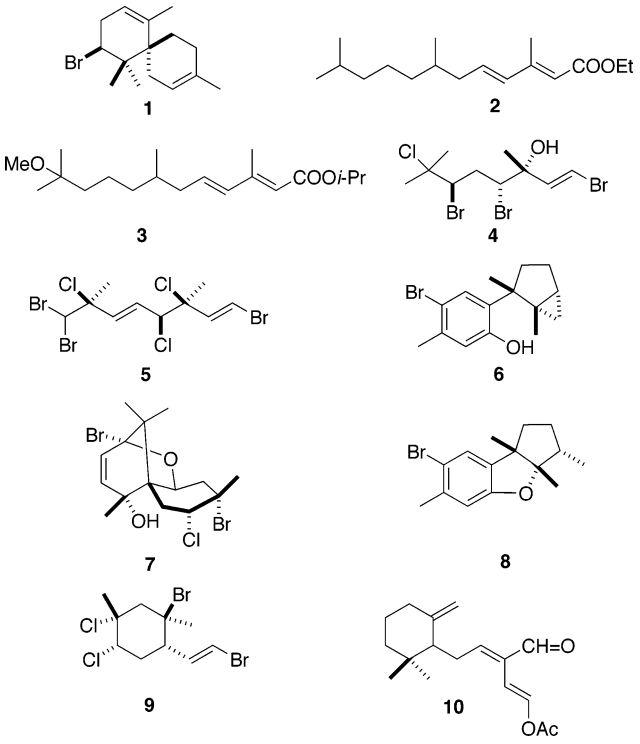
Michael R. Petersen	Ph.D.	1971	The Claisen Rearrangement and its Application to the Synthesis of *trans*-Trisubstituted Olefinic Bonds: the Synthesis of Squalene and Insect Juvenile Hormone
Martha Ophelia Stallard	Ph.D.	1974	Chemical Constituents of the Sea Hare *Aplysia californica*
Raymond John Andersen	Ph.D.	1975	Chemical Studies of Primitive Marine Organisms: Porifera and Bacteria
Jon Stuart Mynderse	Ph.D.	1975	Halogenated Monoterpenes from *Plocamium cartilagineum* Dixon and *Plocamium violaceum* Farlow
Lawrence Evans Wolinsky	Ph.D.	1976	Biomimetic Approaches to Marine Natural Products (Synthesis of 10-Bromo-alpha-chamigrene)
Chris Michel Ireland	Ph.D.	1977	The Chemistry of Some Opisthobranch Molluscs
Stephen Jay Wratten	Ph.D.	1978	Secondary Metabolites of *Chondria californica, Ulosa* sp., and *Pseudaxinyssa pitys*
Douglas Edward McIntyre	M.S.	1979	The Isolation of Biologically Active Compounds from Marine Invertebrates

period proved to be seminal works in marine natural products and set the future direction of the field.

Reading John Faulkner's pedigree and early CV, three things are abundantly clear. First, he was exceptionally well trained as an organic chemist; second, he was very bright; and third, he knew absolutely nothing about oceanography or marine organisms. Thus, being a bright person John turned to what he knew best—organic synthesis. John's first independent publication with a student was on the utility of the Claisen rearrangement for generation of *E*-trisubstituted double bonds.[4] John and his first student, Mike Peterson, used this methodology in the synthesis of *Cecropia* juvenile hormone[5,6] and the marine natural product squalene.[7] John's fifth student, Larry Wolinsky, continued the synthesis legacy although he ultimately proved to be the last student in John's lab to present a thesis focused completely on synthesis. It is also noteworthy that Larry was the first graduate student from the UCSD Chemistry Department to join John's group. Larry's thesis focused on biomimetic approaches to the synthesis of 10-bromo-α-chamigrene (**1**), one of the simplest members of a growing family of halogenated chamigrenes that were being isolated from the red alga *Laurencia*.[8,9] To support their claims that the synthetic route was in fact biomimetic, Larry prepared deuterated intermediates and performed doping experiments to demonstrate that these intermediates were produced by the alga, essentially doing the first biosynthesis experiment in marine natural products.[10]

One of John's first collaborations at Scripps was actually a consequence of the Claisen condensation synthesis project. John's interest in juvenile hormone (JH) and the synthetic analogues ZR-512 (**2**) and ZR-515 (**3**) developed by Zoecon as potential insecticides led to a collaboration with Professor William A. Newman. Professor Newman, a marine biologist at Scripps, studied the endocrine biology of cirriped crustaceans, the marine analogues of insects. The team of Faulkner and Newman and their respective students Chris M. Ireland (John's sixth student) and Edgardo D. Gomez demonstrated that ZR-512 had a profound effect on the developmental biology of the barnacle *Balanus galeatus*. Treatment of barnacle larvae at ppb concentrations of ZR-512 caused the larvae to undergo premature metamorphosis to what appeared to be normal adults with the exception that they never attached to a substrate.[11] John enjoyed saying, "an unattached barnacle is a dead barnacle",[11] and this initial result spawned a number of derivative projects including a study of the potential of JH mimics as antifouling agents.[12]

Martha Stallard, John's second student, was the first student to focus solely on marine natural products. She pioneered studies with the sea hare *Aplysia californica* and its food sources, the red algae *Laurencia pacifica* and *Plocamium cartilagineum*, that ultimately became a mainstay of John's early natural products program. Martha's observations that the chemistry of *A. californica* was highly variable with halogenated monoterpenes such as 7-chloro-3,7-dimethyl-1,4,6-tribromo-1-octen-3-ol (**4**)[13] and (3*R*,4*S*,7*S*)-*trans*,*trans*-2,7-dimethyl-1,8,8-tribromo-3,4,7-trichloro-1,5-octadiene (**5**)[14] dominating in collections where *P. cartilagineum* was abundant and sesquiterpenes such as laurenterol (**6**) and pacifenol (**7**) in areas where *L. pacifica*[15] was abundant provided a scientific rationale and foundation for subsequent studies in the Faulkner lab on the interaction of a wide variety of carnivorous opisthobranch molluscs with their dietary organisms. Martha also provided the first evidence that molluscs could biotransform metabolites sequestered from a dietary source by demonstrating the in situ conversion of laurenterol to aplysin (**8**) in the digestive gland of *A. californica*.[16] The initial studies of *P. cartilagineum* were expanded by Jon Mynderse,[17] who also showed that a second species, *Plocamium violaceum*, produced a family of cyclized, rearranged monoterpenes such as (1*R*,2*S*,4*S*,5*R*)-1-bromo-*trans*-chlorovinyl-4,5-dichloro-1,5-dimethylcyclohexane (**9**).[18]



Ireland continued Stallard's work on sea hares, subsequently showing that the sea hare *Dolabella californica* also sequestered and concentrated algal metabolites but preferentially from brown rather than red algae.[19,20] He also expanded the studies of molluscs to include other

Figure 1. Defensive milk is secreted from glands located around the periphery of the mantle of the mollusc *Onchidella binneyi* after challenge.

herbivores, in particular the saccoglossans, which sequester viable chloroplasts from a green algal source.[21] This initial work led to independent studies by Ireland and Scheuer that showed that saccoglossans are capable of in situ photosynthesis of secondary metabolites.[22] Ireland also provided one of the first examples of an opisthobranch that deployed a glandular defense secretion. In response to challenge, the mollusc *Onchidella binneyi* expels a milky secretion from apical pores situated around the edge of the mantle (Figure 1). This secretion contains a single toxin named onchidal (**10**).[23] Onchidal displayed very potent antibiotic activity and ultimately was discovered to be a potent inhibitor of acetylcholine esterase.[24]

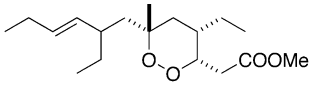
Although Professor William H. Fenical of Scripps has lately shown us the rich and wonderful world of the chemistry of marine microorganisms, John Faulkner was actually one of the earliest investigators of the chemistry of marine bacteria. In the mid-1970s, John, together with his students Raymond J. Andersen and Steven J. Wratten, reported antibiotic compounds from *Chromobacterium*[25] and *Pseudomonas*[26] bacterial strains. Ray and Steven were also the first students in the Faulkner lab to investigate the chemistry of marine sponges, which launched the most productive chapter of John's career. In particular Andersen's studies of the sponge *Verongia*[27] and its associated nudibanch *Tylodina fungina* were a prelude of things to come as; ultimately, the chemistry of sponges and their interactions with other invertebrates and microorganisms became the heart of John's program. Wratten also expanded chemical investigations in the Faulkner lab to other invertebrates including metabolites of the sea pen *Ptilosarcus gurneyi*.[28] Doug McIntyre, the last student in the 1970s generation, finished out the decade with a study of the sponge *Reniera*.[29]

Although this section focused on the studies of John and his graduate students in the 1970s, it should also be acknowledged that John trained a number of postdoctoral fellows during that period including David Vanderah, B. N. Ravi, and Martin Higgs.

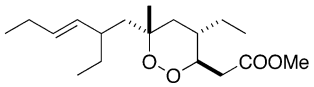
'Do Something Marine'—the 1980s

Having established tenure at Scripps Institution of Oceanography, John Faulkner expanded his involvement in marine natural products and developed a prodigious rate of publication. Some would say that John, who was gaining respect as a dedicated scholar in the field, also progressed from Young Turk to *enfant terrible*. Upon meeting someone for the first time, John was sometimes known to evince an inflammatory statement then observe and evaluate how the individual responded (not always gracefully!). Although John could be provocative, few could fault him for his adherence to a set of high scientific standards that shaped his work and benefited those individuals receptive of his mentorship. John and Paul Scheuer (University of Hawaii) were known rivals, and it is precisely because their formative works often concerned the same subjects. As with all scholarly rivalries, the spirit of competition can also hone intellectual skills and even improve the quality of science, which benefits both competitors and followers alike. It can even provide entertainment. Some of us can recall the 1986 Gordon Conference in Marine Natural Products in Oxnard, California, where an extraordinary scheduling had placed Paul Scheuer as the discussion leader for the session where John would speak. "*John Faulkner is a man who needs no introduction*", Paul deadpanned, in his characteristic baritone Germanic accent, "*but I wouldn't miss it for the world...*".

The first paper of the 1980s published from the Faulkner lab was "Metabolites of the Marine Sponge *Plakortis zygompha*",[30] coauthored with Bindganaval Ravi (one of only two people who have the distinction of having worked with both John and Paul). A second paper on a related topic—metabolites of *P. halinchondroides* with postdoc Don Stierle—appeared later that year.[32a] *Plakortis* spp. produce a suite of polyketide cyclic peroxides of the 'plakortin family' (*cf.* plakortin, **11**), related to the cyclic peroxide chondrillin,[31] and a series of heterocycles (e.g., **12**—**14**), some of which appeared to be rearrangement products of



11 *plakortin*



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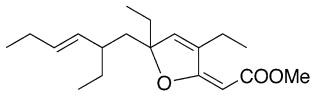

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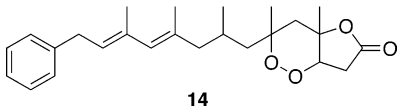

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11, which was reported earlier by Faulkner and Higgs.[32b] Although the papers were a few of many that appeared that decade that described routine characterizations of new marine metabolites, they exemplified the crisp, clean prose and economy of words that characterized John's scientific writings and critical analysis that was reliant upon chemical degradation and precise interpretation of NMR chemical shift data.

If there was an underlying theme to John's papers in the 1980s, it was a preoccupation with metabolites of marine sponges and those things that ate them. Of the 107 papers that John coauthored from 1980 to 1989 (Figure 2), 52 described new compounds from marine sponges and another 14 reported metabolites from dorid nudibranchs—spongivorous (sponge-eating) marine molluscs. Nudibranchs are shell-less molluscs with superficial resem-

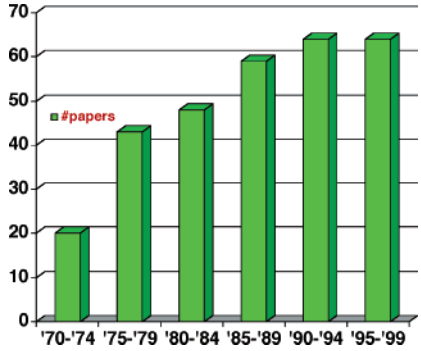
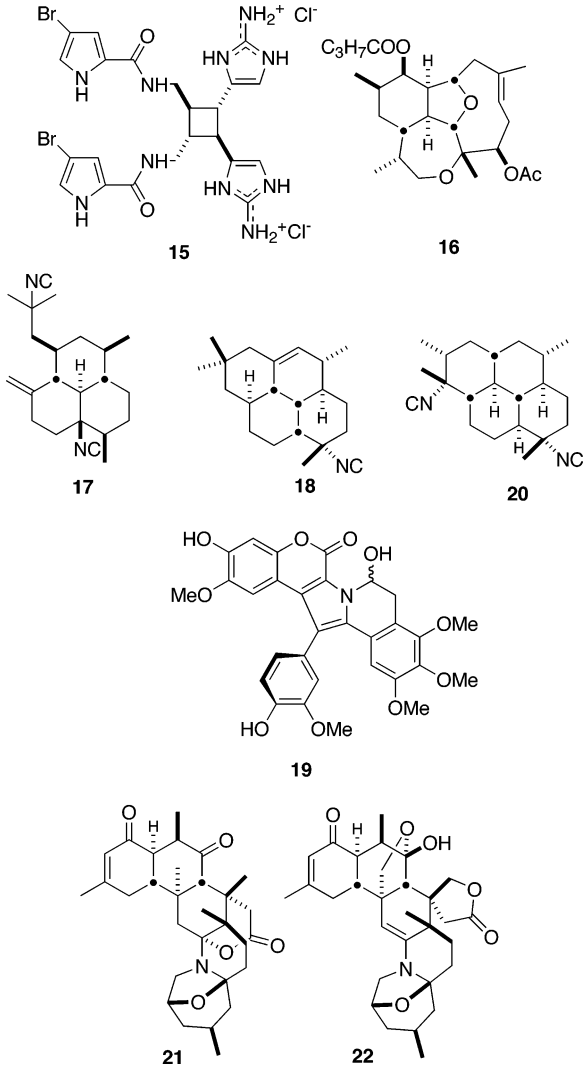


Figure 2. Papers coauthored by D. John Faulkner from 1973 to 1999. John began collaborating with Bob Jacobs (UC Santa Barbara) during this time (6 papers) and had already collaborated extensively with Jon Clardy (then, at Iowa State University and, later, Cornell University). From 1973 through 1989, Clardy and Faulkner coauthored 46 papers and many more after.

blance to garden slugs except for two anatomical features that are obvious even to the casual observer: the presence of a large branchial plume—an external gill or 'naked lung'—adapted to a marine environment (terrestrial slugs are pulmonates that breathe through a mantle cavity) and brilliant, varied body coloration and patterns that range from the subtle and discrete to spectacular and downright outlandish. The chemical constituents of many nudibranch metabolites were recognized by Scheuer, Cimino, Faulkner, and others to be of sponge origin but assimilated by these molluscs from a diet sponge, each restricted or specialized to one or a few particular sponge species. It seemed that nudibranchs purposefully assimilated sponge natural products into specialized glands, and it was assumed they were then deployed to deter predators, much as *Onchidella* exuded the bitter substance onchidal (**10**) when challenged.

In John's lab, two graduate students, Roger Walker and Jill Hochlowski, took the challenge and investigated species of dorid nudribranchs of the genus *Chromodoris* from La Jolla and the Sea of Cortez, Mexico, as did Janice Thompson and Brad Carté, who expanded studies of the chemistry and ecological implications of natural products to other molluscs. With rare exception, the structures of natural products derived from sponges embodied carbon skeletons that were without precedent among the natural products of terrestrial plants or microbes. These were exciting times for a young academic finding new opportunities on the shores of a vast ocean of discovery. Like a curious child at the beach who is compelled to explore the tide pool by upturning every rock or shell, John found that the allure of discovery—a new alkaloid or a novel polyketide—was irresistible. In particular, the attendant scientific 'detective story' of spectroscopic structure elucidation strongly appealed to John.

Perhaps John's most fruitful collaboration was with Jon Clardy, who was at the time tenured at Cornell University and also collaborated with Paul Scheuer and other investigators in marine natural products. Jon's research group had contributed X-ray crystallographic analyses of dozens of marine natural products. From 1973 and throughout the following decade, Faulkner and Clardy coauthored over 45 papers on the structures of unusual metabolites from marine sponges, algae, cnidarians, and molluscs. Many of the structures that emerged from these studies, such as the brominated pyrrole sceptrin (**15**),[33] asbestinin-1 (**16**),[34] the polycyclic isonitrile **17**,[35] from the Caribbean sponge *Hymeniacedon amphilecta*, **18** [compounds **17** and **18** are *cis*-fused analogues of the marine isonitrile diisocyanoadociane (**20**) (Baker, J. T.; Wells, R. J.; Oberhänsli, W. E.; Hawes, G. B. *J. Am. Chem. Soc.* **1976**, *98*, 4010−4012)] from a Palauan *Halichondria* sp.,[36] the atropisomeric lamellarin A (**19**) from the prosobranch *Lamellaria* sp.,[37] and a new alkaloid class, represented by zoanthamine A (**21**)[38] and zoanthenamine (**22**),[39] from an Indian *Zoanthus* sp., are familiar representatives of the unique chemistry of marine natural products from sponges and continue to attract interest today from synthetic chemists and chemical ecologists, alike.



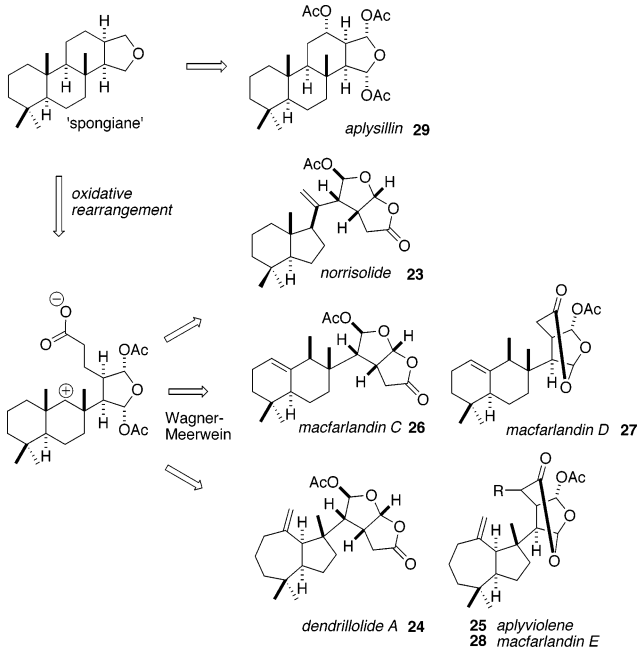
New compounds appeared with nearly every species examined, and rivalry for precedence of new findings was palpable. The pace of research accelerated in the Faulkner lab, quickened by the exhilaration of discovery and the push to reach further into the new realm of natural products. These were heady times in a competitive arena, and the search for compounds was propelled by industrious graduate students, such as Brian Sullivan, Roger Walker, Jill Hocklowski, and Brad Carté (Figure 3). In such times, one also occasionally tasted the bitterness of being 'scooped' by competitors who secured claim to the structure by publishing first. Yet civility could prevail when the right thing to do was publish together. One paper from this era, "Sesquiterpenoid constituents of eight porostome nudibranchs",[40]—in fact the only paper that bears the names of both Paul Scheuer and John Faulkner on the author byline—exhibited the virtue of sharing credit.

Much of this work was published in *Journal of Organic Chemistry*, which reflected the fascination of organic chem-

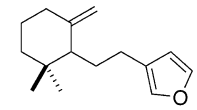
Mary Kay Harper	PostDocs
Grad. Students	Don Stierle
	Peter Djura
Jill Hocklowski	T. Nakatsu
Roger Walker	Ellen Barrabee
Janice Thompson	Kim Albizati
Brian Sullivan	Richard Rosser
Brad Carté	Peter Kay
Mike Kernan	Robert Capon
Joe Pawlik	Tadeusz Molinski
Denise Manker	Diana Kushlan
Haiyin He	Eoin Fahy
Steve Bobzin	Andrea Stierle
Barbara Potts	Katarina Kassülke
Mia Unson	Fan Hua

Figure 3. The 1980's team; students and postdocs from the Faulkner lab, 1980−1989.

ists with each new unusual terpenoid structure that came to light and the hint of an underlying pattern in the biosynthesis and ecology of the marine organisms that produced them. For example, the rearranged diterpenes norrisolide (**23**)[41] from the nudibranch *Chromodoris norrisi*, the related dendrillolide A (**24**)[42] from the Palauan sponge *Dendrilla* sp., aplyviolene (**25**) from an Australian *Chelonaplysilla* sp.,[43] and macfarlandins C (**26**) and D (**27**)[44] from a local nudibranch, *C. macfarlandi*, collected near Scripps (La Jolla, CA) all had structures that implied a common precursor: the hypothetical diterpene 'spongiane', which had been proposed earlier on the basis of the discovery of aplysillin (**29**) from *Aplysilla rosea*[45] and related compounds. With remarkable insight, John proposed a unifying biosynthetic hypothesis[42a] that linked the *seco*-ring B spongiane diterpenes **23**−**28** with **29** and predicted the existence of other, as yet undiscovered *seco*-spongianes. The subject of spongiane diterpenes, their chemistry and distribution between dictyoceratid sponges and the nudibranchs that prey upon them, continued to draw John's interests throughout the decade.[46]



The research emphasis on sponge−nudibranch associations was largely driven by findings in chemistry, but questions pertaining to the chemical ecology of sponges and nudibranchs were at the heart of the matter. An often-cited hypothesis, that nudibranchs sequestered metabolites from specific sponge species selected in their diet to gain defense against fish predators, was supported by experiments in the Faulkner laboratories. The natural products found in *Cadlina luteomarginata* (**30**−**32**), and acquired from various identified dietary sponge species, inhibited feeding by fish when applied in food pellets at 10−100 μg/mg, concentrations that are similar to those found in the mollusc.[47]



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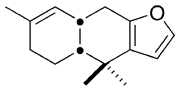

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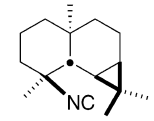

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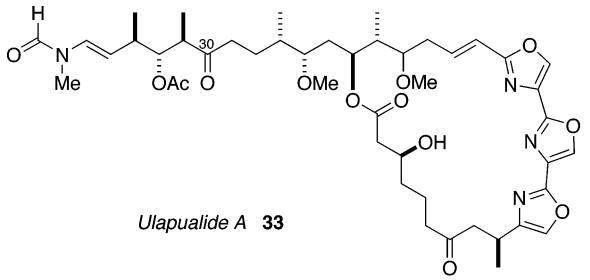
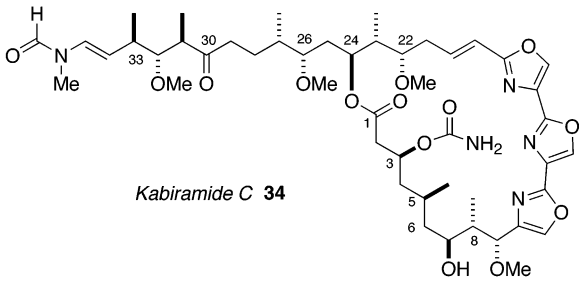
In 1983 John coauthored a paper with Michael Ghiselin,[48] an evolutionary biologist (California Academy of Sciences) in which they supported the hypothesis that shell-*less* molluscs evolved from their shelled ancestors after acquisition of unpalatable chemicals accompanied by conspicuousness through bright warning coloration (aposematism). They argued chemical defense was preadaptative: the protective shells, no longer essential, became vestigial and were eventually lost as the molluscs evolved with a reliance on chemical protection that made possible their soft-bodied existence in a predatory environment. Modern-day shell-less nudibranchs clearly assimilate repugnant chemicals from their diet, but it was not yet obvious that these same chemicals could protect the progeny of nudibranchs—their eggs.

In 1986, Fusetani and Scheuer independently disclosed the first examples of the extraordinary 'trisoxazole macrolides'. The two compounds—ulapualide A (**33**)[49] and kabiramide C (**34**),[50] with unprecedented structures containing three contiguous 2,4-disubstituted oxazole rings—were obtained from the gelatinous pink egg masses laid by the giant Indo-Pacific nudibranch *Hexabranchus sanguineus*, the Spanish Dancer.[51] At about the same time, at Scripps, Mike Kernan was pursuing his Ph.D. degree with John and working on the structures of different trisoxazoles, halichondramide (**35**)[52] and dihydrohalichondramide (**36**), obtained from a tropical north Pacific sponge *Halichondria* sp. Molinski, a postdoc at the time in the Faulkner group, was independently isolating **34** and a new compound, tetrahydrohalichondramide (**37**), from extracts of whole *H. sanguineus* and its egg masses that were collected from the U.S. military base Kwajalein Island in the Northern Pacific.[53] (It's interesting that both Kernan and Molinski worked, at first unbeknownst to each other, on essentially the same compounds—one set from *Halichondria* sp., the other from *Hexabranchus sanguineus*—despite being in adjacent laboratories in the Faulkner group.) As events transpired, a fascinating connection between the sponge, its predator *Hexabranchus sanguineus*, and its progeny was revealed. Joe Pawlik, who was working on his Ph.D. thesis under John and had

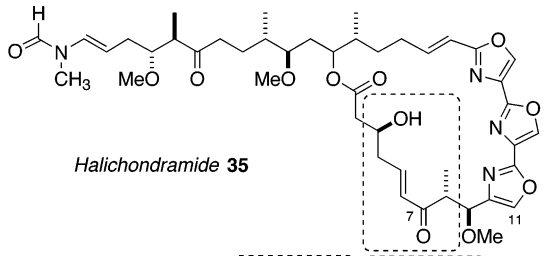
brought back the specimens from Kwajalein, took an interest in the developments in chemistry of *Halichondria* and *Hexabranchus*. He undertook a series of elegant experiments with Mary Kay Harper[54] that demonstrated several principles: *Hexabranchus sangineus* held in captivity fed exclusively on the *Halichondria* sp. and acquired **35** and **36** (but not **37**) from the sponge. The latter



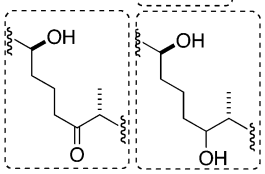
Ulapualide A **33**



Kabiramide C **34**



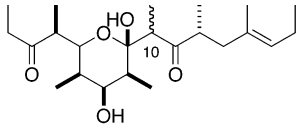
Halichondramide **35**



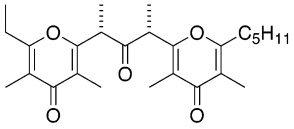
36 **37**

compound appeared to be synthesized de novo by the nudibranch from the former substances assimilated from its sponge diet. The compounds were powerful feeding deterrents. Food pellets treated with any of the trisoxazole compounds inhibited feeding by the common reef fish *Thallasoma lunare* at concentrations as low as 0.01% w/w (100 ppm). Last, the trisoxazoles were found to be concentrated approximately 10-fold within the egg masses laid by the nudibranch, which suggested that the compounds were deployed to protect the eggs from reef predators including fish. Thus, potent antifeedant metabolites, acquired by *Hexabranchus sanguineus* from a specialized sponge diet or metabolized from dietary compounds, conferred significant advantages to the individual and its progeny. This appears to be a general strategy in other marine invertebrates. For example, Lindquist has investigated distribution of metabolites within ascidians during their various stages of life and found that eggs or larvae are also often imbued a suit of protective natural products from the adult parents.[55]

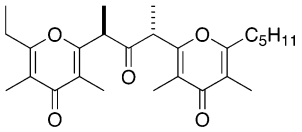
Interest in other molluscs was expanded in the late 1980s through examination of the metabolites of Siphonaria. Unlike nudibranchs, siphonaria have developed a mantle cavity (pseudobranch) that allows the mollusc to breathe air when exposed at low tide or function as a gill while underwater. Despite their protective shells, siphonarids elaborate a series of polyketide metabolites, which may protect them from predators when exposed above the tide line. Jill Hochlowski isolated denticulatins A (**38**) and B (**39**) from *Siphonaria denticulata*,[56] while Denise Manker, Ph.D. student, identified maurapyrones A (**40**) and B (**41**) from *S. maura*,[57] collected in Costa Rica, and vallartanones A (**42**) and B (**43**)[58] from the same species collected



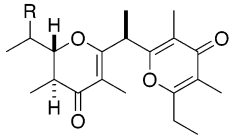
38 (10*R*) *denticulatin A*
39 (10*S*) *denticulatin B*



40 *maurapyrone A*



41 *maurapyrone B*



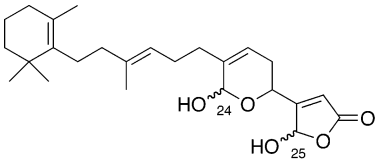
42 R = CH_3 *vallartanone A*
43 R = H *vallartanone B*

in Puerto Vallarta, Mexico. Polypropionates are rare and found elsewhere only among the fermentation products of certain *Streptomyces* species (e.g., erythromycin). Denise, in collaboration with Mary Garson (then at University of Wollongong, Australia), demonstrated de novo biosynthesis of denticulatins in *S. denticulata* using in vivo incorporation experiments with sodium [^{14}C]-propionate.[59] Thus, it appears that *Siphonaria* spp. are capable of producing their own secondary metabolites, unlike nudibranchs, which must obtain them from the things they eat.

Most of the work in John's lab during the 1980s was funded by the National Institutes of Health (NIH), the National Science Foundation, and, later, the California Sea Grant program. The justification of NIH funding, like those of many investigators of the day, was the search for antibiotic substances (antibacterial and antifungal) and evaluation of compounds in simple but effective agar disk diffusion assays. The search for antibiotic natural products matured into more focused investigations of pharmacologically active substances from marine sponges toward the end of the decade. In the 1980s, Robert Jacobs (UC Santa Barbara) formed successful collaborations with John Faulkner and William Fenical to study anti-inflammatory compounds. All three groups set about to discover anti-inflammatory compounds from marine invertebrates and algae, mainly by testing pure compounds isolated in the labs at Scripps and sent to Jacob's laboratory at UC Santa

Barbara. Among the many interesting findings from this program was the discovery of the potent anti-inflammatory activity of manoalide (**44**).[60]

Manoalide was actually first isolated by Paul Scheuer's group from the tropical Northern Pacific sponge *Luffariella variabilis* and reported with modest antimicrobial activity.[61] Jacobs revealed that manoalide was a potent and *irreversible* inhibitor of phospholipase A_2 (PLA_2) from beevenom and other sources, including human synovial fluid. PLA_2 is a component of the eicosanoid pathway that is responsible for release of cytokines (e.g., prostaglandins, leukotrienes) that are associated with pain and inflammation. The search for PLA_2 inhibitors, funded now by California Sea Grant (and later Allergan Pharmaceuticals in Irvine, CA), turned up other classes of compounds with comparable or lower activity, but also a series of manoalide derivatives that exhibited *reversible* inhibition against the target enzyme. Many collections of *L. variabilis* were made during this period, and as the species name implies, the sponge was not always easy to identify in the field. A pilot extraction of one pooled collection of over 100 kg of sponge gave no pure **44**, but an inseparable mixture of this compound and luffariellin A (**45**), together with B (**46**). A



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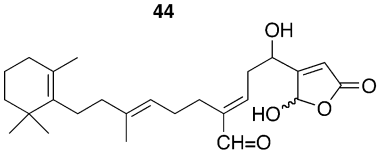

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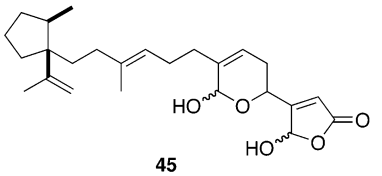

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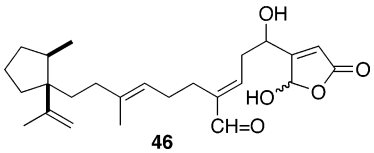

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brief investigation revealed that some pieces of sponge tissue in the collection contained only **45** and **46**, but no **44**, while others only **44** and *seco*-manoalide (**47**) or a mixture of luffariellins. While this suggested the interesting possibility of at least two different 'chemical races' of *L. variabilis*, the practical task at hand was that each of the hundreds of pieces in the collection had to be individually analyzed. Since HPLC methods were not suitable for analysis (the compounds spontaneously epimerize at C24 and C35, leading to poor retention characteristics), the entire collection was examined laboriously by extraction and ^{1}H NMR measurements of each piece, which were then pooled accordingly. In the paper published in *Journal of Organic Chemistry*, John mentions, almost in passing, "*of the 410 specimens of* L. variabilis *examined by* ^{1}*H NMR spectroscopy*", which attests to a certain persistence in keeping to the task at hand. (Actually, it attests to the persistence of John's technician at the time, Joaquin Caso, a brave individual who completed the task and purified gram quantities of manoalide for further pharmacological evaluation.) This phase of research culminated in a deeper understanding of the chemical properties of **44** and identification of the PLA_2 pharmacophore. John's graduate students, Mike Kernan and Barbara Potts, together with the Jacobs group, demonstrated the involvement of the masked 1,7-dialdehyde present in **44** with key lysine side chain residues of the PLA_2 as the basis of the enzyme inhibition.[62]

The level of sophistication of pharmaceutical discovery in John's research program expanded with key collaborations with industrial partners, including Eli Lilly (Indianapolis), Smith Kline Beecham (King of Prussia, PA), and Bristol Myers Squibb (originally at Groton, CN, and later at Princeton, NJ). The latter association was consolidated with William Fenical, Yuzuru Shimizu (University of Rhode Island), and Jon Clardy in a partnership that was awarded one of the first National Cooperative Drug Discovery Grants issued by the NIH in the late 1980s. John's program gained strength from this NIH grant, which was renewed twice, and continued to fund John's research for the remainder of his career. John, long an advocate for academic collaboration with biologists and chemists alike, coauthored many papers with, among others, C. B. Rao (Andhra University, India), Mary Garson (University of Queensland), Kim Albizati (Wayne State University), and Ray Andersen (University of British Columbia), who had returned to Scripps for a sabbatical.

Following his review on marine natural products in *Tetrahedron* (published in the *Tetrahedron Report*s series No. 28 under the unassuming title "*Interesting Aspects of Marine Natural Products Chemistry*"),[63] John was approached by the Royal Society of Chemistry to write significant and comprehensive reviews on the field. In 1984 John published the first of what would become a series of 17 reviews for *Natural Products Reports* on new marine natural products.[64] The series would become one of the most authoritative and widely referenced bodies of work in natural products chemistry.[65] In the first year, John actually wrote two reviews[64p,q]—one covering natural products from algae and the second review covering metabolites from invertebrates and other organisms—but in each subsequent year both topics were combined into a single paper. John worked punctually on his material. Those who were in John's group at the time knew that every Friday afternoon he would disappear into the Scripps library to pore over new journal issues, gather citations, and copy papers that he would diligently file and download into a proprietary computer database he had designed himself. The reviews were well received by readers from the beginning and, in fact, achieved a celebrity status of sorts when the Institute of Scientific Information declared the 2000 issue a 'hot article' due to the exceptional number of citations it attracted.[66] When asked about the success of his works, John demurred, "*I suppose that the reviews in this series are so highly cited because they enable an author to cite a single source rather than a long list of original papers...Perhaps best of all, it provides a comprehensive list of references, including some rather obscure journals.*" He was particularly grateful for the high quality of the Scripps library collection and the dedication of its staff. "*I must ...acknowledge the important contribution made by the librarians and the inter-loan department at Scripps*".

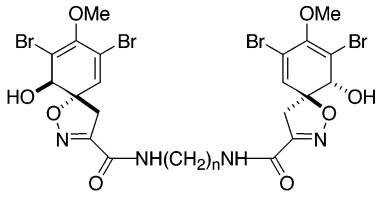
At the close of the 1990s John was asked to write a 'millenium review' of what he considered to be the most important developments in marine natural products between 1972 and 1999.[67] Among other topics, he chose to

acknowledge the roots of the science in plant natural products. "*It is clear that the early directions taken by marine natural products chemists drew as much from the examples provided by insect chemical ecology as from the longer history of phytochemistry... It is the integration of the three fields of study that has given marine natural products chemistry its unique character and vigour...*"

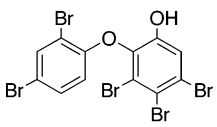
Delving Deeper—Location, Location, Location

By the end of the 1980s, the field of marine natural products had matured. Heading into 1990, some 6000 novel compounds had been isolated from marine organisms spanning the major marine invertebrate phyla.[64k] Clearly the 'low-hanging fruit' had been picked, and shifts in methods and focus were palpable. Marine natural products chemists were turning to previously uninvestigated organisms for new sources of chemistry, with particular emphasis on marine microorganisms. In terms of isolation chemistry, groups were hotly pursuing previously intractable secondary metabolites including water-soluble compounds and those present in vanishingly small amounts. The latter was largely made possible through tremendous advances in technology, including NMR spectroscopy, mass spectrometry, and isolation and purification techniques. The field advanced to embrace these changes, and a new area of research in the field of marine natural products was emerging simultaneously. As more and more complex and interesting structures were being elucidated, the number of secondary metabolites closely resembling those from terrestrial microorganisms or phylogenetically distant invertebrates was increasing in parallel.[68−70] Sponge-derived natural products proved to dominate these seemingly anomalous phylogenetic findings, which drove researchers to speculate with increasing frequency on a microbial source of many of the natural products they were finding. However, experimental support for these suppositions was lacking at the time, and experimental methods to investigate these questions had yet to be established.

To rationally delve into the question of bacterial origins of secondary metabolites, particularly in sponges that are sometimes seen to harbor enormous populations of bacteria, Faulkner maintained several unwritten, but wholly logical, rules when seeking out a new system (=invertebrate and prokaryotic symbiont). After meeting the first requirement that the same or very closely related compounds be found in different taxa, the following questions were asked: Are the compounds similar in structure to known microbial natural products? Is the microbial population large enough to support production of a compound? Is the microbial population consistently present with the chemistry being studied? And do the probable biosynthetic pathways suggest a microbial source (for example polyketide or nonribosomal peptide biosynthetic pathways typical of microorganisms versus terpene biosynthesis typical of sponges)? Thus, symbiosis-related projects soon became multifaceted ones and prospered from the already established tradition in the Faulkner group of the intermeshing of natural products chemistry with fieldwork and careful observation. To embark on the area that captivated John Faulkner's attention through the next two decades, he and his students started with elegant localization studies with the marine sponge *Aplysina fistularis* (=*Verongia thiona*). *A. fistularis* produces several 3,5-bromotyrosine-derived metabolites, including aerothionin (**48**) and homoaerothinin (**49**) in high yields.[71,72] Taking advantage of the unique emission properties of bromine, Janice Thompson and coworkers used energy-dispersive X-ray microprobe analysis to neatly demonstrate that these brominated metabolites



aerothionin n=4 **48**
homoaerothionin n=5 **49**



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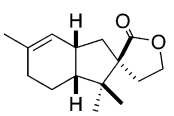
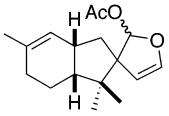


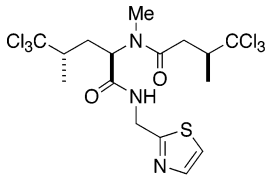
51 *herbadysidolide*



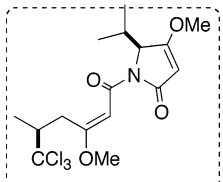
52 *spirodysin*

were localized to sponge cells, spherulous cells in particular, in *A. fistularis*.[73] Though this system was not one in which bacterial symbionts were implicated, it was the first time that any marine secondary metabolite had been localized to a cellular or subcellular level and thus became the groundwork for upcoming localization studies, each of which required the use of a variety of techniques, often utilized in unorthodox ways.

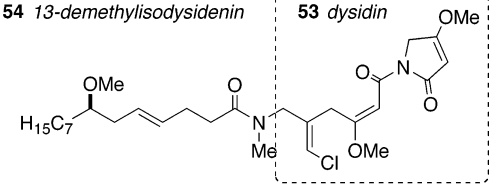
The first system chosen for study in which microbial symbionts were implicated in the production of sponge-derived metabolites was that of *Dysidea herbacea*, a marine sponge found in the Indo-Pacific. *D. herbacea* had the distinction of having been extensively studied by marine biologists and ecologists owing to its symbiosis with the cyanobacterium *Oscillatoria spongeliae*,[74] which can occupy as much as 50% of the sponge volume. Assorted chemicals had been isolated from extracts of *D. herbacea* including brominated polybromo-biphenyl ethers, such as **50**,[75,76] sesquiterpenes such as herbadysidolide (**51**)[77] and spirodysin (**52**),[78] and polychlorinated amino acid-derived metabolites such as dysidin (**53**)[79] and 13-demethylisodysidenin (**54**).[80] Dysidin and analogues sparked the interest



54 *13-demethylisodysidenin*



53 *dysidin*



55 *malyngamide A*

of many because a closely related compound, malyngamide A (**55**),[81] had been found in free-living cyanobacteria. To determine the source of some of these metabolites, Mia Unson first investigated the origins of the polychlorinated compounds in a sponge chemotype containing the chlorinated compounds along with the terpenes **51** and **52**. Like most sponges, the cells of *D. herbacea* and its associated organisms can be dissociated by subjecting finely divided pieces to calcium/magnesium-free seawater (Figure 4). Upon dissociation of cells from the tissue of *D. herbacea*, Unson exploited the photosynthetic pigments present in *O. spongeliae* and cleanly sorted sponge cells and cyanobacteria on the basis of fluorescence using flow cytom-

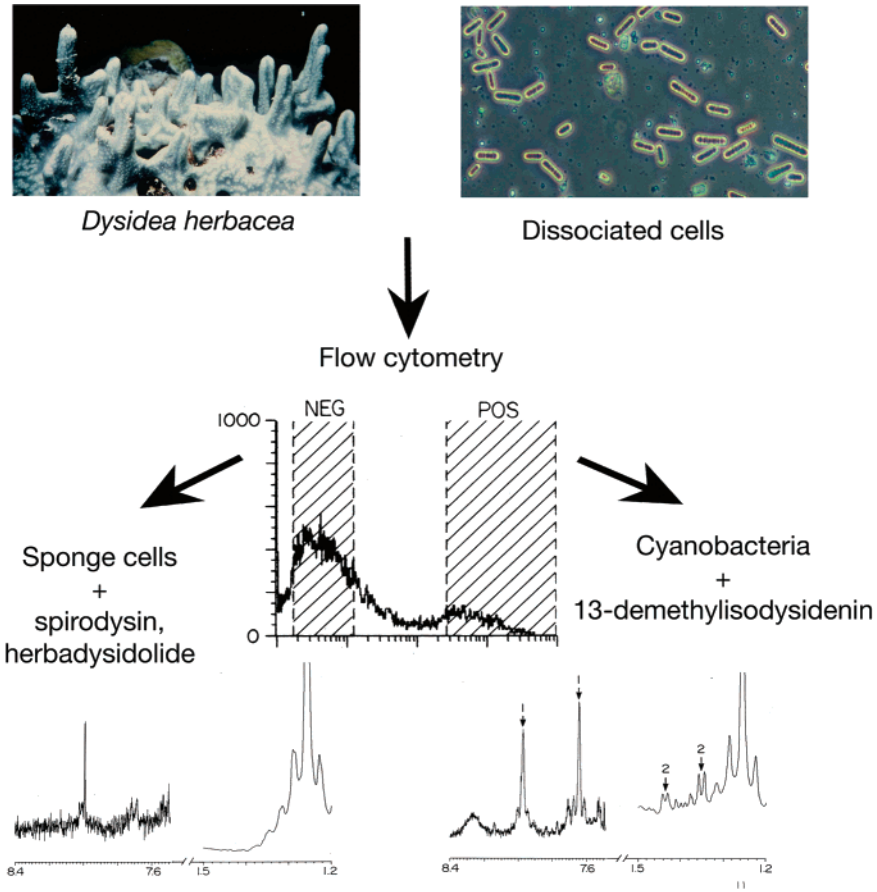


Figure 4. Cell separation scheme used by Unson et al. for localization studies with *Dysidea herbacea*.[82,83]

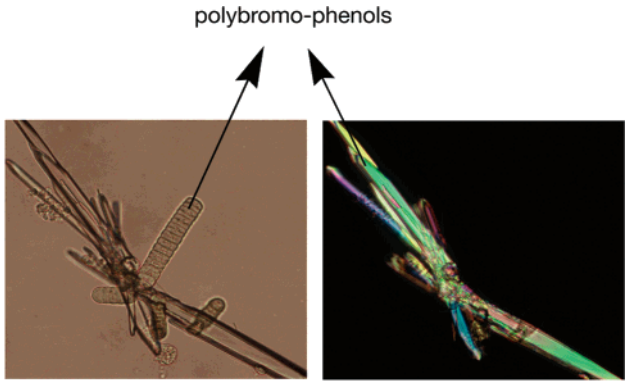
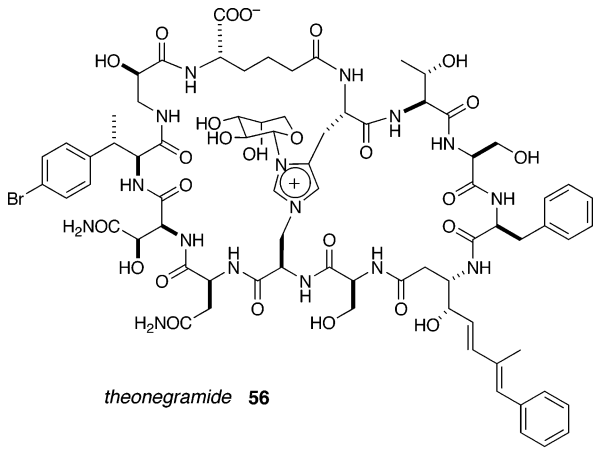


Figure 5. Light and phase contrast micrographs of crystals of polybromophenol **50** and a cyanobacterial filament found in *Dysidea herbacea*.[82]

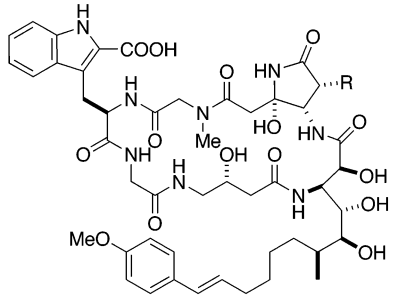
etry.[82,83] NMR and mass spectral analyses of these two fractions showed the terpenes to be present exclusively in the sponge cell fraction and the acylated polychlorinated amino acid peptide **54** in the cyanobacteria. This was the first demonstration that secondary metabolites ascribed to a marine sponge are localized in prokaryotic symbiont cells.

Unson next tackled localization studies in a different chemotype of *D. herbacea* that contains polybromo-biphenyl ethers as the major secondary metabolite as well as large populations of filamentous cyanobacteria. While preparing cells for flow cytometry and microscopy, Unson made the startling observation that large crystalline needles were present in all sample preparations, including the intact sponge (Figure 5).[84] This observation was unexpected given that *D. herbacea* lacks a spiculose skeleton of silica or other crystalline material. The X-ray fluorescence spectrum of the organic-soluble crystals revealed that the crystals were indeed the polybromo-phenols, and chemical analyses demonstrated that these compounds were confined to the cyanobacterial cells. Thus, not only were the compounds localized in the cyanobacterial symbionts, it appeared that the cyanobacteria were synthesizing and excreting these compounds in such high levels (up to 12% of the wet weight) that they crystallized within the sponge body.

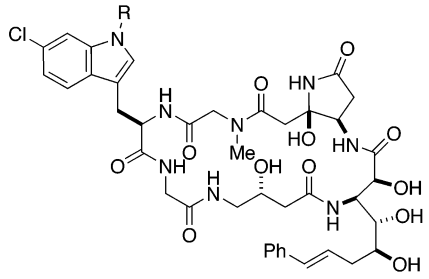
Largely influenced by the accomplishments of Nobuhiro Fusetani and Shigeki Matsunaga, members of the Faulkner laboratory spent the next few years isolating and elucidating the structures of very complex cyclic peptides, glycopeptides, and glycopeptidolipids. Coincidentally, all of these complex peptides came from sponges belonging to the polyphyletic order Lithistida and included theonellamide[85] derivatives theonegramide (**56**)[86] and theopalauamide,[87] microsclerodermins A−D (**57**−**60**),[88,89] aciculitins A−C (**61**−**63**),[90] and mozamides.[91] Many of these peptides contained unprecedented amino acids or unusual amino acids identified previously in secondary metabolites from prokaryotes only, namely, eubacteria and cyanobacteria.[92] One lithistid sponge in particular, *Theonella swinhoei*, had been the subject of intense speculation because it contained not only peptides with β-amino acids similar to those found in cyanobacterial metabolites, but also polyketides, such as swinholide A, that previously had been limited to cyanobacterial secondary metabolites such as scytophycin C.[93] Thus, several independent groups speculated on a bacterial origin for both the peptides and polyketides that often coexisted in *T. swinhoei*, backing up their suspicions with scanning electron micrographs showing the presence of filamentous bacteria. Carole Bewley completed a survey of *T. swinhoei* specimens collected around the globe and found theonellamide-like peptides, swinholide A, and filamentous bacteria to coexist.[94] Unlike *D. herbacea*, however,



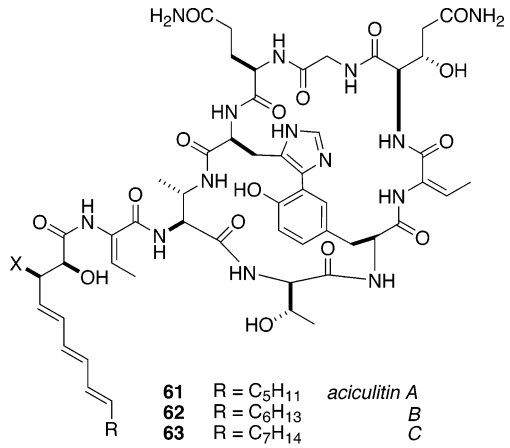
theonegramide **56**



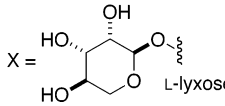
microsclerodermins A **57** R = OH
B **58** R = H



C **59** R = $CONH_2$
D **60** R = H



61 R = C_5H_{11} *aciculitin A*
62 R = C_6H_{13} *B*
63 R = C_7H_{14} *C*



X = L-lyxose

T. swinhoei specimens contain four distinct cell types including sponge cells, heterotrophic eubacteria, heterotrophic filamentous bacteria, and if exposed to light, unicellular cyanobacteria limited to the exterior of the sponge (Figure 6). Mechanical stress, provided by passage through a juicer followed by differential centrifugation of the cell suspension, led to cell preparations of greater than 90% purity for each cell type. Extraction and chemical analyses of each of these four cell fractions showed the sponge cells and unicellular cyanobacteria to be devoid of any detectable secondary metabolites and the cyclic peptide theopalauamide to be limited to the filamentous bacteria, and swinholide A was found in a mixed population of eubacteria present in the sponge.[95] Thus, in this study, diverse bacterial populations, at least one of which can be considered symbiotic, were shown to be entirely responsible for the production of secondary metabolites arising from two unrelated biosynthetic pathways.

The secondary metabolites associated with *Dysidea* spp. and *T. swinhoei* so closely resembled other natural products biosynthesized by cyanobacteria that these systems begged to be studied first. There still remained numerous other fascinating examples of structurally similar secondary metabolites, distributed across different phyla, many of which possess useful biological activities. One such example was provided by the aromatic alkaloids known as pyridoacridines, compounds that had been isolated from sponges, tunicates, molluscs, and cnidaria.[96] Continuing to forge new ground in this area, Christine Salomon embarked on localization studies of the pyridoacridine dercitamide (**64**) in the sponge *Oceanapia sagittaria*.[97] Salomon and coworkers cleverly took advantage of the inherent chemical properties of the natural product itself, namely, its pH-dependent color change and intrinsic fluorescence, and localized dercitamide, not to resident bacteria, but to sponge cells. By utilizing sophisticated visualization techniques, including confocal, epifluorescence, and transmission electron microscopy, they succeeded in localizing the compounds not just to separated cell fractions, but also in sections of the intact sponge itself (Figure 7).

The studies outlined here are but highlights of Faulkner's contribution to the study of symbiosis as it pertains to the production and origin of marine natural products. There is no question that, cumulatively, John Faulkner and his students laid the groundwork for others to begin to study the chemical phenomena associated with symbiosis, and this work will continue to have a significant impact on the field for years to come. These studies overcame the technical hurdles needed to study chemical symbioses, providing researchers with a toolbox of approaches to begin looking at a system of interest. They provided the first strong evidence that microbial symbionts do in fact produce sponge-associated secondary metabolites, and perhaps most importantly, they helped launch the now burgeoning field of marine natural product biosynthesis, which in marine systems encompasses studies of microbial symbionts and whole systems alike.

Eric Schmidt (contributing his own original work in this memorial issue) was the first of Faulkner's students to initiate the requisite molecular biology and phylogeny studies that accompany investigations into symbiosis when he used 16S rRNA gene sequencing to characterize the filamentous bacterial symbiont of *Theonella swinhoei* that produces the antifungal peptide theopalauamide.[98,99] Schmidt et al. assigned the bacteria to a novel group of δ-proteobacteria, naming the symbiont *Candidatus* Entotheonella palauensis. Expanding from there, Schmidt proceeded to examine, in concert, both the chemistry and phylogeny of *T. swinhoei* filamentous bacterial symbionts collected from many sites around the globe, proposing for

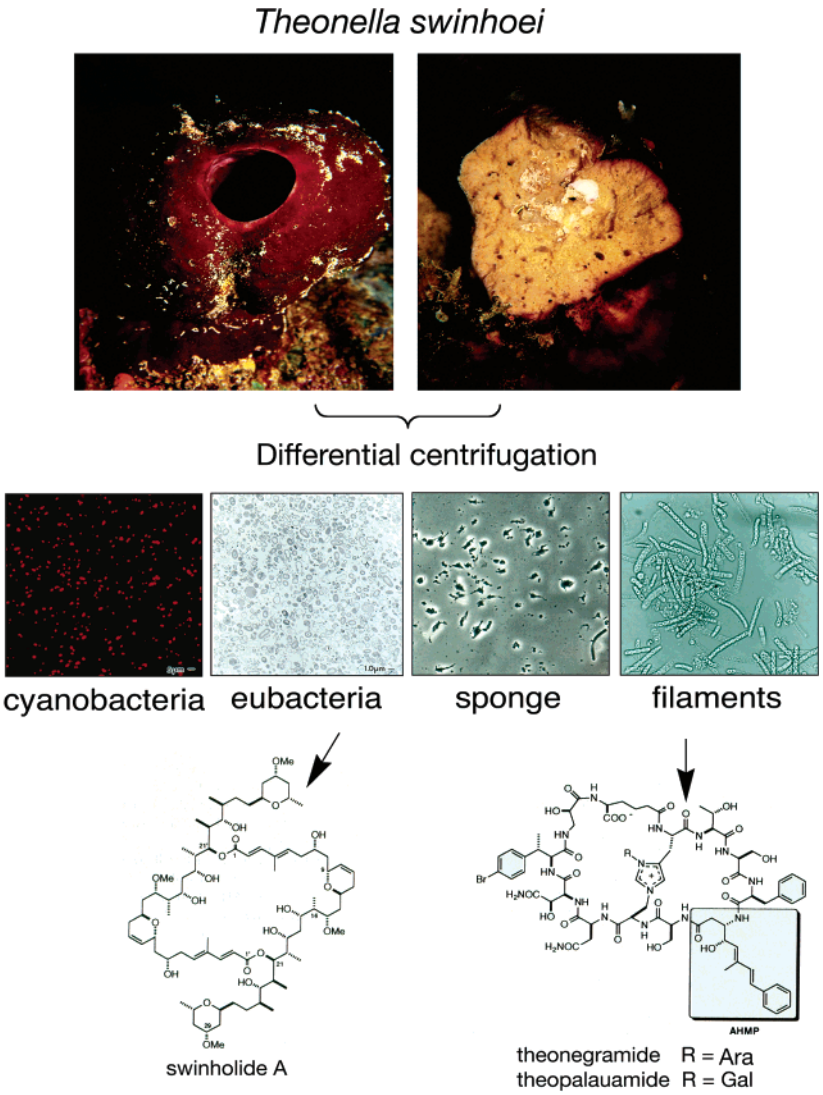


Figure 6. Underwater photographs of the interior and exterior of *Theonella swinhoei*, and electron and light micrographs of the four types of cells, after cell separations, located in *T. swinhoei*.[94,95]

the first time that it may be possible to cooperatively predict chemistry and phylogeny within certain symbiotic systems.

The groundwork laid by Faulkner's graduate students, postdoctoral associates, and collaborators both in industry and in academia led to important advances in understanding the nature of secondary metabolite structure, localization, and biosynthesis. At the close, John had published over 350 papers during the span of a remarkable career. There was, of course, the human element in these achievements that bonded members of John's group in each successive generation of students and postdocs.

One function of an academic scholar is to eventually replace him- or herself. John's 'academic progeny' have followed from his lead and gone on to independent careers. It may not be fully recognized that John Faulkner was often an enthusiastic promoter of young scientists by fostering those who followed him in academic paths.[5] (Aside from the authors, the following have launched their own independent scholarly careers with an emphasis on marine natural products: Joseph Pawlik (Professor, University of North Carolina, Wilmington), Eric Schmidt (Assistant Professor, University of Utah), Robert Capon (University of Queensland, Director of the Centre for Molecular Biodiversity).)

Epilogue

"I do not know what I seem to the world...I seem to have been only a boy, playing on the sea-shore...finding a smoother pebble or prettier shell...while the great ocean of truth lay all before me"—Isaac Newton

Newton was actually paraphrasing a predecessor of his—the great writer and fellow Englishman, Milton—who then weighed in on academics (as many do now) with ruminations on the value of things that preoccupy their time.

"...Deep versed in books...collecting toys and trifles for choice matters, worth a sponge...As children gathering pebbles on the shore..."—Milton, *Paradise Lost*

Indeed, 'choice matters, worth a sponge'! The field of marine natural products has given much to the scientific world, and John has given much to the field. It can be too easy to evaluate these contributions in terms of a compendium of new chemical structures when they are much more. Marine natural products have stimulated numerous new investigations in other disciplines, which may not otherwise have been possible. John's legacy belies the words of Milton by showing how the dedication and persistence of a disciplined mind, directed by curiosity toward a nascent and unexplored field of research, can lead to paths of adventure, scientific discovery, and enduring truth. John's career, like the marine natural products he worked with,

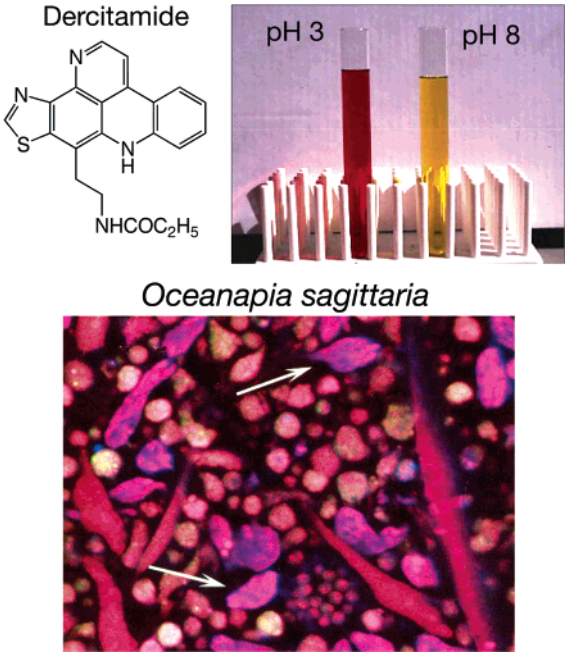


Figure 7. Studies of *Oceanapia sagittaria* and the pyridoacridine dercitamide (**64**). Upper right panel illustrates pH-dependent color change of **64**, which Salomon et al. used to localize the compound in sponge cells with the aid of confocal microscopy.[97]

has transcended its origins by enlivening scientific inquiry through interdisciplinary research and creation of opportunities that nurture the greater good.[100]

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Supporting Information Available: Complete bibliography of published works by D. J. Faulkner (1962−present). This material is available free of charge via the Internet at http://pubs.acs.org.

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