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Announcement

Ulrich Matern: 2008 Recipient of the PSNA Phytochemistry Pioneer Award

Natural product biosynthesis: My first encounters.

After completing the Staatsexamen in Pharmacy at the University of Freiburg in 1969, I was looking for a research position in either industry or university, rather than pursuing a business as a licensed pharmacist. At that time, the Faculty of Biology had just moved into a new modern building placed in the enchanting Freiburg Botanical Gardens. While most departments of the faculty had broadly dedicated their research to either light regulation, such as phytochrome signaling, phototropic microalgae or cyanobacteria, an Institute of Plant Biochemistry (IPC) was also created, chaired by the irrepressible Hans Grisebach who was already widely known for his flavonoid research. Grisebach had pursued some of his studies in Melvin Calvin's laboratory and had also extensively used radiotracers for biosynthetic studies.

I applied to the IPC as a PhD student, and was immediately accepted, but, to my surprise, was asked to work on the biosynthesis of branched-chain carbohydrates which occur predominantly in antibiotics. This was Professor Grisebach's second major field (other than flavonoids). At the time, I did not realize the difficulties in growing the exotic strains of *Streptomyces* that were stored in sealed ampules in the fridge for many years; nor did I envisage the analytical problems posed by minute quantities of strange sugars. A long period of trial and failure thus ensued. However, with a stay of several weeks in the laboratory of Hans Zähner, an authority in the field of antibiotics at the University of Tübingen, this finally resulted in the ability to grow the bacteria which were supposed to produce quinocycline antibiotics.

In the end, both my perseverance and failure tolerance paid off! Minute quantities of two tridesoxyoctoses were isolated from these antibiotics in the form of their methyl glycosides, and the incorporation of label from acetate/pyruvate into their two carbon side-chains was demonstrated.

Yet, there was another hurdle to overcome at that time: the acquisition and interpretation of ^{1}H NMR spectra which required the most sophisticated spectroscopic equipment not yet available in Freiburg. The Fourier transform technique was then in its infancy; however, some measurements were kindly acquired overnight for us on one of the first 100 MHz Fourier-NMR spectrometers at Ciba-Geigy AG, Basel – provided there was no thunderstorm

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within a 100 km distance! Fortunately, Bruker-Physik, Karlsruhe, also later offered some help, and the studies were successfully completed.

After receiving my PhD in biochemistry in 1972, this line of research continued for a while and was extended to enzymatic investigations on dihydrostreptose/streptose biosynthesis. Despite all tasks being accomplished, this difficult topic and the slow progress were somewhat frustrating. Anyway, I was more interested in plant secondary products! At about this time, the group of Klaus Hahlbrock in the neighboring laboratory began to intensify their research on parsley cell cultures following an observation made by chance: induction of flavonoid biosynthesis by light irradiation. As a consequence, large batches of parsley cells were needed for enzyme isolation, and my experience with 20 l fermenters of bacterial batch culture was helpful to develop a protocol for large-scale plant cell fermentation. This led to joint publications on enzymes of flavonoid biosynthesis, i.e. malonyltransferases that acylate flavonoid glucosides to their hemiesters or the corresponding esterases, and of regulation of the pathway(s) by light.

An excursion into plant pathology: The next chapter

Both microbial and plant research meet in the field of plant pathology, and the biochemistry of plant-pathogen interactions began to receive increasing attention about 30 years ago. Gary Strobel at Montana State University in Bozeman, a leading figure, had just published a highly recognized account of the binding of *Helminthosporium* toxin in sugar cane; I later joined him as a visiting postdoctoral research associate. The time in Montana was rewarding in many ways: Gary opened my eyes not only to the relevance of host-specific and non-host-specific phytotoxins in plant diseases, but also for their potential applications. I enjoyed his unconventional intelligent approaches to new research topics and his engaging personality. At the same time, Montana surprised me with its marvelous landscape, and Gary was a great guide and mentor for numerous outdoor activities. During my time there, a collection of potato plants regenerated from cells of a single leaf was grown for the first time in the field, and they revealed an unexpected variance in morphology. Using a partially purified toxin preparation from *Alternaria solani*, the predominant agent of blight disease in potato, we demonstrated that the plants differed considerably in their response to the causative toxin. This phenomenon later became known as ‘‘somaclonal variation”. Studies on another blight disease caused by *Alternaria carthami* in safflower were also initiated in Bozeman and these continued after my return to Freiburg. Properly irrigated safflower as a vegetable oil crop had been rated superior to sunflower, but *Alternaria* blight was also a limiting factor. We discovered that this fungus produces brefeldin A, a 16-membered lactone, and phytotoxic to many plants. It was produced predominantly when the fungus was grown on safflower. Although 14- and 16-membered macrolides were in use as antibiotics worldwide, no respective esterase had been reported until 1985. Our search for such an esterase yielded an enzyme from a strain of *Bacillus subtilis* that was recombinantly produced, crystallized, and analyzed by X-ray diffraction in Zygmunt Derewenda’s lab at the University of Virginia, Charlottesville. Surprisingly, it turned out to be a bacterial homolog of the mammalian hormone-sensitive lipase. The gene may be used to generate safflower transformants with enhanced resistance to *Alternaria* blight, but the proof of principle still remains to be established. *Alternaria* blight diseases were chosen early on as research issues in view of the few fungal diseases reported from the *Apiaceae*, such as parsley.

Elicitor-induced phenylpropanoids in Freiburg: Another journey

After my return to Freiburg, I focused on phenylpropanoids whose formation could be induced in cell cultures of parsley and bishop’s weed (*Ammi majus* L.), as well as from additional members of the Apiaceae upon addition of fungal elicitors, i.e. cell wall extracts from either *Alternaria* or *Phytophthora* ssp. Linear furanocoumarins (psoralens) and other derivatives of cinnamic acids were thus isolated as phytoalexins, and the enzyme activities forming psoralen and bergaptol from umbelliferone *in vitro* were soon characterized. This subject was studied further and resulted in numerous publications up to the present time. However, another project emerged during the course of these studies and received priority status for some years: the plant cells turn brown upon elicitation and accumulated cell wall esters which yielded *p*-coumaroyl- and feruloyl-type compounds on hydrolysis, i.e. ferulic acid, methoxytyrosol and vanillin, although the activity of caffeic acid *O*-methyltransferase (COMT) activity remained at an insignificant level. This phenomenon finally led to the discovery and isolation of *S*-adenosylmethionine:caffeoyl-CoA *O*-methyltransferase (CCoAOMT). The gene was cloned, functionally expressed in *E. coli*, and found to be essential *in planta* for the methylation of the caffeoyl moiety. CCoAOMT occurs ubiquitously in plants and was shown, in collaboration with Joseph Varner’s group at Washington University St. Louis, to be involved in lignification. This has since considerably affected the textbooks on the ‘‘metabolic grid” of lignification.

It is remarkable that only a few phytoalexins contain nitrogen, and these are derived primarily from anthranilic acid. As an example, carnations were reported to accumulate dianthalexins by *N*-acylation of anthranilate with either hydroxybenzoyl- or cinnamoyl-residues and by further modification. A distinct *N*-acyltransferase was isolated from elicited carnation cells catalyzing both hydroxybenzoylation and cinnamoylation steps with the corresponding CoA-esters as acyl donors. The cDNA cloning and functional expression then yielded the first example of the superfamily of enzymes, later termed BAHD transferases. The research on anthranilic acid derivatives was continued by cDNA cloning of acridone synthase (ACS) from *Ruta graveolens*. ACS is a polyketide synthase extending the anthranilic carboxyl by six carbons (requiring three malonyl-CoAs) followed by cyclization. This also brought us back to flavonoid biosynthesis, because of the obvious parallels in the mode of action to that of chalcone synthase (CHS).

Marburg and molecular plant research in Marburg

I accepted the chair of the Institute of Pharmaceutical Biology at the Philipps-University Marburg some 13 years ago. During this time, the molecular studies on acridone alkaloid biosyntheses were extended. We also picked up on the threads of our former coumarin research and chose to investigate the 2-oxoglutarate-dependent dioxygenases in flavonoid biosynthesis as another issue. Major advances were attained in all of these projects. The ACS pivotal in acridone alkaloid formation displayed narrow substrate specificities using *N*-methylanthraniloyl-CoA as a starter substrate, but did not produce ‘derailment’ products. This differed from the *in vitro* activity of CHS, although the sequences of ACS and CHS showed a high degree of identity. The sequence motifs responsible for ACS vs. CHS activity were therefore studied using site-specific mutagenesis, and CHS was mutated to develop some ACS side activity. Furthermore, the *N*-methyltransferase acylating anthranilate, but not anthraniloyl-CoA, was characterized and functionally

expressed in *E. coli*. This DNA provided a likely tool to shunt anthranilate from either primary metabolism or to block the pathway in acridone-producing plants. Acridone alkaloids were produced exclusively in plants of the Rutaceae, and cloning of the N-methylanthranilate: CoA-ligase would enable the transformation of unrelated plants to accumulate acridone.

Concerning furanocoumarin biosynthesis (cf. Phytochemistry 2007, 68:1830-3), the mechanism of the pivotal psoralen synthase was described in collaboration with Wilhelm Boland's group, Jena (MPI for Chemical Ecology). This P450 monooxygenase was unique, because it catalyzed an oxidative carbon-carbon bond cleavage reaction by a syn-elimination releasing acetone. The coding DNA was cloned from *Ammi majus* in cooperation with the group of Fréderic Bourgaud, INRA Nancy, as the first coumarin-specific gene. More recently, the analogous gene for the angular branch pathway, encoding angelicin synthase, was also cloned from *Pastica sativa*. Overall, these studies have revealed many fundamental data on the biochemistry and genetics of furanocoumarin formation and now provide a solid basis for future regulatory and ecotoxicological studies. Our interest in 2-oxoglutarate-dependent dioxygenases (2-ODDs) arose because their mode of action resembled that of P450 monooxygenases and because four 2-ODDs catalyze crucial steps in the biosynthesis of flavonoids. Predominantly flavone synthase I (FNS I), flavonol synthase (FLS) and anthocyanidin synthase (ANS) were studied. A number of reports that resulted from these studies described the cDNA cloning, the substrate and product specificities, as well as the effects of mutagenesis. Most notably, the assignment of ANS function still poses a problem: this enzyme was multifunctional *in vitro* showing FLS and other activities, and the exact role in proanthocyanidin formation was under debate after a puzzling dimerization reaction observed with (+)-catechin as substrate. Thus, we contributed a little to refine the pattern of flavonoid biosynthesis, but there remains much work to those who follow up.

I retired in spring of 2008 as determined by current governmental policy. Looking back on so many years of natural product research, and having gone through stages of pure analytical work and extensive enzymological studies to molecular biology, I am grateful for all the opportunities I have had to explore biosynthetic pathways. However, the modern world of 'chips' and prefab 'molecular kits' seems to better suit the next generation of scientists. I am passing on the torch, even though there remains plenty to do in the fields of coumarins, acridone alkaloids, and flavonoids; these have all been under study in many laboratories worldwide for more than half a century. On reflection, I have had a very productive time, and hope that those that follow will build on this foundation.

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