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The recent recognition by the PSNA of my pioneering career in phytochemistry may indicate pioneering genes, if such exist, in my genome. These would date from the early nineteenth century when my great-great-grandparents emigrated from Belfast to the wilds of what was then Upper Canada, about 45 km north of Lake Ontario, and literally carved a home and farm out of the forest primeval. If so, these genes have been expressed on several occasions during my career.

My interest is established early: origin of the methyl group of nicotine

My interest in phytochemistry took root shortly after the Second World War at Michigan State, where I was Richard U. Byerrum's first graduate student, doing a Master's thesis on a seed enzyme. Biochemistry was then taught as a predominantly static subject, and I recall my excitement at first reading Baldwin's *Dynamic Aspects of Biochemistry*, the first text I had seen that examined in detail the many biochemical pathways of microbial and animal metabolism. By the time I had to choose a PhD thesis topic, Dick Byerrum had become quite keen on plant metabolism, and in fact studied it for the rest of his career. I decided to work under him on a plant biosynthetic pathway. As biosynthesis in plants was a subject in its infancy, this was pioneering work, but the elegant groundbreaking tracer studies on photosynthesis by Melvin Calvin's group were pointing the way to the future in this area, and I was eager to get on board. We decided to seek evidence for transmethylation in plants, a reaction then only recently established in animals, and began studies with ^{14}C on the origin of the methyl group of nicotine in tobacco, my one and only foray into alkaloids. We found its methyl carbon to originate from the methyl carbon of methionine, demonstrating this reaction in plants for the first time.

Lignins in Saskatoon

In 1948 the National Research Council of Canada had opened its Prairie Regional Laboratory (PRL) in Saskatoon, Saskatchewan, for the announced purpose of research in chemurgy, the development of new industrial products from agricultural wastes, and such research was indeed done for over 30 years. But NRC's president was Ned Steacie, and PRL's director Aleck Ledingham, both of whom most wisely allowed their subordinates a very long leash, and two of the PRL sections were headed by the rising young carbohydrate chemist Raymond Lemieux,

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and prominent microbial biochemist Arthur Neish, who both had strong commitments to basic research. When in 1953 Lemieux's group achieved the first chemical synthesis of sucrose, emphasis on fundamental investigations was firmly established. About 1950, one of Lemieux's chemists, John Stone, had begun preliminary work on the biosynthesis of lignin with $^{14}CO_2$, probably owing to a perceived chemurgical connection, and they decided that to continue they needed a plant biochemist. I was appointed to that position in 1951, a green PhD and the first plant scientist to join the PRL. Little did anyone then appreciate, certainly not I, the pioneering significance of that appointment: it was the beginning of a metamorphosis that would result in the complete conversion of the institution to plant science by the early 1980s, when the PRL became the Plant Biotechnology Institute (PBI). When Stone left the PRL not long afterwards I continued the lignification work, which was moved under Neish's wing. But I also digressed briefly into the carbohydrate field, studying the formation of cellulose from ^{14}C-glucose and working out procedures for the chemical degradation of glucose and xylose to determine ^{14}C distribution.

Cambridge interlude

In 1955 I began a year's postdoctoral leave in Sir Alex Todd's laboratory in Cambridge, to enhance my knowledge of organic chemistry. I was assigned to work not in his major area of nucleotides but on the structure of aphins, pigments of red aphids. Involved as well in this thankless project was another of Todd's postdocs, Eddie Haslam; thus of all those who made a career in phenolics research, Eddie is my earliest acquaintance. Todd was sidetracked in this aphin research, seeking evidence for a postulated structure that later proved incorrect. I once took a solution of the pigment to London trying to get a spectrum by the new technique of NMR on one of Varian's demonstration instruments. We failed on that try, but it was this approach, combined with the success by another member of the group in cleaving the pigment into two identifiable fragments, that led later to the eventual elucidation of the correct structure.

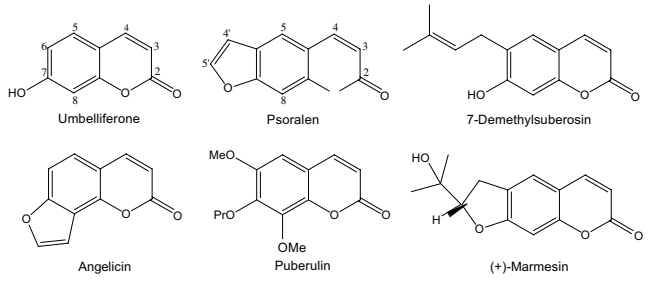
Back to lignification

Back in Saskatoon, seeing that I had been making progress on the lignin front, Art Neish, too, had begun to feel the excitement of phytochemical research. He switched fields, and with Ledingham's support persuaded the NRC to establish a new plant biochemistry section at the PRL and to extend the facility to accommodate it. Neish was the first head of this new section, which made a number of important contributions to several areas of phytochemistry. Among other interests he collaborated with me on lignin biosynthesis for about four years, and we established that lignins were derived from shikimic acid, the first demonstration of its role in plants. (In the early sixties Neish moved to the NRC's Atlantic Regional Laboratory in Halifax, where he studied the biochemistry of ocean plants until his untimely death at 57 from cancer in 1973.) Also working with me on lignification, for a very productive 2 years, was Takayoshi Higuchi who, after his return to Japan, won highest honours for many distinguished contributions to lignin chemistry and biochemistry.

My group worked on the reactions between carbon dioxide and the coniferyl and sinapyl alcohols found in Karl Freudenberg's Heidelberg laboratory to be the building blocks of lignins, seemingly polymerizing randomly. We established with ^{14}C radiotracer techniques the reaction sequences involving phenyl- and 4′-hydroxy-phenylpyruvic acids, phenylalanine and tyrosine, cinnamic acid, and cinnamic acids oxygenated variously at ring positions 3, 4 and 5, that led eventually to Freudenberg's lignin building blocks. Work about this time in Eric Conn's laboratory, where enzymes then identified as phenylalanine and tyrosine ammonia lyases were discovered, filled in major missing steps of these pathways. We had noted species differences in the utilization of phenylalanine and tyrosine, with grasses alone having the ability to convert the latter to lignin. This was explained by the finding that tyrosine ammonia lyase activity, now believed to be localized on the same protein as that against phenylalanine, is confined almost exclusively to grasses.

Interest in coumarins

In the late fifties I had begun to investigate the biosynthesis of coumarin and its 7-oxygenated derivatives structurally based on 7-hydroxycoumarin, umbelliferone. (For structures of coumarins see figure.) This subject had hardly yet been explored, although there had been preliminary studies by both Conn and a German group of which Heinz Floss soon became a part and made important contributions. Neil Towers, then at McGill University, spent the summer of 1958 with me and we made a good start studying coumarin itself, which actually commonly occurs bound as a glucoside in intact cells. Later I studied lavender, which elaborates both coumarin and the *O*-methylated umbelliferone, herniarin. ^{14}C feedings indicated a pathway branched at cinnamic acid: its *o*-hydroxylation leading to coumarin and *p*- to umbelliferone, via 2,4-dihydroxycinnamic acid, and by implication to its vast range of derivatives. Umbelliferone can thus be considered the potential precursor of almost all coumarins, as only a few lack 7-oxygenation.



A new society is born: PSNA (Phytochemical Society of North America)

Memorable during this period was an overland journey in 1961 with five of my PRL colleagues to Fort Collins, Colorado, where I gave a paper in a symposium Gestur Johnson had organized on plant phenolics. It was during this meeting that the Plant Phenolics Group of North America, the PSNA's precursor organization, first saw the light of day, with Simon Wender as its first president, and Vic Runeckles, who probably more than anyone else can be regarded as the instigator of the PPGNA, as secretary-treasurer, a post he held for five years. I served as its third president, and the PSNA honored me with a life membership in 1981.

A move to academe

My next pioneering venture came in mid-1964, when I left the PRL to return to my home province of Ontario and join the faculty of Trent University, being newly built from scratch in Peterborough. It was an exciting challenge, but an ultimately rewarding one, as this institution before long built itself a high reputation for teaching, and became the most research-intensive of the predominantly undergraduate Canadian universities. However, the move inevitably meant a setback in my research, as for over 2 years I had neither the time nor any laboratory facilities. It soon became evident that in such a new and small institution there would not be, at least in the short term, the luxury of conducting research on a variety of projects, and that my program would have to be more selective. I therefore chose to abandon studies on the exasperatingly amorphous lignins in favor of the beautifully crystalline coumarins. Fortunately others with more persistence later probed lignification to increasing depths, with the major advances made by Norman Lewis and his associates continuing to be of great significance.

Furanocoumarins to the fore

When we had permanent laboratory facilities at Trent I was able to get back to serious work on coumarins, now with increasing attention to the furanocoumarins occurring particularly in the Umbelliferae and the Rutaceae. We at Trent, with several other new Ontario universities, had been allowed only limited graduate studies – a grievous handicap. To this day the department offers no independent PhD program and I never had a PhD student, but as I did have a succession of postdoctoral fellows and visiting scientists, it was possible to make some reasonable progress. As well, soon after moving I collaborated very successfully for several years with a young phytochemist, Warren Steck, who had taken my place at the PRL, and who in the 1980s became the first director of the newly formed PBI.

Warren and my group, which then included visiting professor Mohammed El-Dakhakhny from Alexandria, continued tracer experiments on pathways to several coumarins. We examined principally the linear furanocoumarins, based on psoralen, in *Ruta graveolens* and *Pastinaca sativa*, but also did limited studies on their angular isomers, based on angelicin, in *Heracleum lanatum*. Both types were formed from umbelliferone, and we found the isopropyldihydrofuranoid compounds marmesin (the (+)-isomer) and columbianetin to be the respective intermediates, clearly suggesting prior isoprenylation at positions 6 and 8 of umbelliferone, respectively. Subsequent tracer work indicated that 6-dimethylallylumbelliferone, demethylsuberosin, was intermediate between umbelliferone and marmesin, and the angular analogue osthenol between umbelliferone and angelicin. Evidence also surfaced that in the furanocoumarins further ring oxygenation follows elaboration of the furan ring. After I had spent a year's sabbatical with John Staba in Minneapolis to study cell culture techniques, some of this work was done with the use of *R. graveolens* cell cultures by Douglas Austin, a postdoc from Glasgow.

On this basis enzyme investigations were undertaken. In the early seventies Brian Ellis, who had joined me for a year's postdoctoral study, identified and characterized the dimethylallyl transferase of *R. graveolens* that substituted a five-carbon prenyl sidechain at the 6-position of umbelliferone, the first step in the pathway from this general precursor of complex coumarins to the linear furanocoumarins. Daljit Dhillon further purified and characterized this enzyme, and found evidence for a chloroplast association. I had also gotten back to my PhD thesis area, trans-methylation, and my colleagues Joan Thompson and Satish Sharma identified and characterized the methyl transferases mediating the formation of the 5- and 8-methoxypsoralens bergapten and xanthotoxin. We completely separated these two transferases on affinity columns through Satish's expertise in this technique, deriving in the process important information on the reaction mechanism – a compulsory-ordered sequence in which the enzyme first binds to the methyl donor *S*-adenosyl-methionine, inducing a binding site for the specific phenolic substrate.

A break for writing: the natural coumarins

Jesús Méndez of Santiago de Compostela in Spain had worked with me for a year investigating pathological aspects of phenolic metabolism in tomato plants with crown gall tumors. In the late seventies, he proposed to organic chemist and coumarins specialist Robert Murray at Glasgow a monograph on the chemistry and occurrence of the natural coumarins. As they thought such a work would benefit by inclusion of biochemical aspects, they asked me to participate. The result, published in 1982, was a Wiley monograph *The Natural Coumarins: Occurrence, Chemistry and Biochemistry*. Although the book enjoyed rave reviews and has proved a very useful reference to specialists, it had disappointing sales – an expensive volume in a potentially very limited market.

Greater simplicity?

Our studies had not neglected the simple coumarins. Many of these are poly-oxygenated in various patterns, raising intriguing speculation about the order in which substitution occurs in the biosynthetic pathways. Evidence has firmly established the precursor role of umbelliferone for this class of coumarins, as for furanocoumarins. I showed that 6,7-dihydroxycoumarin (aesculetin) in chicory and 7-hydroxy-8-methoxycoumarin (daphnetin) in *Daphne mezereum* were derived from this general precursor. Work on this class of coumarins, as on others, encounters the difficulty that many oxygenation patterns are elaborated only by tropical or subtropical species to which access is difficult in icy Canada, absent an established botanical garden nearby. However in the early eighties we collaborated with Douglas Rivett of Rhodes University to study the pathway to a 6,7,8-trioxygenated simple coumarin, puberulin, one of only 22 of this pattern then known to exist, in *Agathosma puberula*, a South African species. Extensive tracer studies coupled with identification of intermediates in extracts by mass spectrometry supported a pathway from umbelliferone successively involving 6-hydroxylation, 6-*O*-methylation, 8-hydroxylation, 8-*O*-methylation and finally 7-*O*-prenylation.

Regretfully I was never in a position to investigate the molecular biology involved and look for the genes corresponding to the known enzymes. By the time this was indicated I was nearing the end of my career, and in addition this type of research was simply not possible in a small university, at least then. This aspect was left for others to pursue.

A new partner and a shift in emphasis

In 1986 I had been joined at Trent by a Polish botanist and cell physiologist with strong phytochemical leanings, Alicja Zobel of Warsaw University, whom I had met at the joint phytochemical meetings organized by Chris van Sumere in Ghent 2 years earlier, and who wanted to spend a year as a visiting scientist in North America, split between my laboratory and Geza Hrazdina's at Cornell. On Halloween of that year she became my wife, and we continued a scientific collaboration that lasted until my retirement from research in 1993. With her botanical background she was interested in localization of coumarins within the plant and the immunological location of their biosynthetic enzymes, areas I had never touched.

One of our first discoveries arose from her investigation of coumarins on leaf surfaces. Work on waxes elsewhere had employed organic solvent extraction, which we tried, to remove surface compounds, but Alicja had an inspiration to test brief extraction with almost-boiling water to melt these surface waxes, and we were astonished to find that this removed orders of magnitude more furanocoumarins than did the solvents. The hot water had released coumarins embedded in the waxy epicuticular layer, but without damaging the cell membranes. In some plants, especially *R. graveolens*, the amount on the surface exceeded that within the cells. This shed new figurative light on the photophytodermatitis long known to be due to contact with linear furanocoumarin-bearing plants followed by exposure to UV radiation. It had always been assumed that the leaves of the plant had to be crushed to produce this dermatitis, but our work showed that, at least in some cases, merely brushing the leaf surfaces is sufficient. Alicja has also obtained preliminary evidence of the toxic principle of poison ivy, erushiol, on the leaf surface. She and I enjoyed a productive 7 years and coauthored 18 publications dealing with various aspects of localization of coumarins, and the influence on it of UV radiation and other environmental factors, including pollution. But as my role in this work was mainly supportive, and she has published reviews covering it, space considerations do not permit me to do so here.

Now that I am long retired I, like probably all researchers, can envisage in hindsight things that I'd have liked to do or should have done. However one has to stop sometime, I maintain my interest in the PSNA, and there is plenty to do in my former fields of research to keep many people busy for a long time to come. I wish them every success!

Stewart A. Brown