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Toxic plants: a chemist's perspective

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Abstract. Chemistry has long been an integral part of toxicology, as the two fields originated in much the same way: the investigation of plants with interesting properties. In this chapter I review the role that chemistry has played in understanding toxic and medicinal plants. After some introductory remarks, three broad areas are addressed: the role of natural products in understanding plant taxonomy and evolution, recent developments in chemical synthesis, especially efforts to discover and efficiently synthesize novel structures based upon naturally occurring toxins, and finally, developments in the new field of systems toxicology, which seeks to integrate all aspects of an organism's response to toxic insult.

Introduction

"*What the eyes perceive in herbs or stones or trees is not yet a remedy; the eyes see only the dross. But inside, under the dross, there the remedy lies hidden.*"
"*Is not a mystery of nature concealed in every poison? What has God created that He did not bless with some great gift for the benefit of man?… In all things there is a poison, and there is nothing without a poison. It depends only upon the dose whether a poison is poison or not.*"

Paracelsus (1493–1541) [1, quoted in 2]

Natural products, the wide range of small molecules extracted from the dross of the biological realm, are the gift to which Paracelsus refers. Natural products are also known as secondary metabolites. They include molecules from plants, as well as those of bacterial, fungal, animal and marine origin. They have played a critical role in modern medicine – a medicine that saves someone from cancer is a poison to the cancer cell, but deliverance for the patient. Newman and Cragg at the National Cancer Institute in the United States have monitored the sources of new drugs over several decades [3]. Over the 25-year period from 1981 through mid-2006, 34% of candidate drug molecules were natural products or were made from natural products (only small molecules considered; vaccines and biologicals excluded). If one adds molecules prepared synthetically, but whose pharmacophores were inspired by natural products, the total is 51%. Among anticancer agents over the period from the 1940s to mid-2006, the numbers are even more impressive, 42 and 56%, respectively. Although few surveys have broken out plant-based substances from the entire spectrum of drug candidates, Butler has recently reported that of 225

natural product-derived drugs in various stages of development, 49% of them are of plant origin [4]. While individual pharmaceutical companies' interest in and emphasis on natural products has varied over the years, it is clear that natural products will continue to contribute significantly to drug discovery and development [5].

The number and diversity of these plant natural products are enormous [6]. These compounds represent investments by the plant in defense against herbivores such as insects and grazing animals, as well as infectious agents like fungi, bacteria, and viruses. In many cases, the compounds also serve communication functions. Defense is necessary due to the sessile lifestyle of plants; escape is not an option. Table 1 gives a sense of the number and variety of structures known. Further, in a plant, the synthesis of a particular molecule is not constant, but varies in a spatial and temporal manner. For instance, defensive compounds are often present in young leaves, but as the growing season progresses and the leaf matures, the type of compounds change. The type of tissue is also important. Reproductive organs such as seeds are frequently well-defended because of their importance to the survival of the organism, while fleshy fruits often have compounds designed to attract animals and ensure their dispersion (the seeds inside the fruit survive the gastrointestinal tract unharmed). Finally, it has recently become apparent that many compounds originally believed to be of plant origin are actually produced by endophytic fungi that live within the plant tissue [7–11]. This appears to be the case with some of our most important anticancer agents, taxol [12, 13], camptothecin [14, 15], and podophylum-derived compounds [16], as well as important herbal medicines like St. John's Wort [17] (Fig. 1).

Table 1. The diversity of natural products

Category		Number
Alkaloids		12 000
Cyanogenic glycosides		60
Phenylpropanoids (incl. tannins, anthocyanins, flavonoids, coumarins, lignans)		6000
Glucosinolates		100
Non-protein amino acids & miscellaneous amines		800
Polyacetylenes, alkylamides, fatty acids & waxes		1900
Terpenes		
C_{10} (monoterpenes)	2500	
C_{15} (sesquiterpenes)	5000	
C_{20} (diterpenes)	2500	
C_{30} (triterpenes)	5000	
C_{40} (tetraterpenes)	500	
	Total terpenes	15 500
	Grand total	43 560

Data adapted from Wink [35].

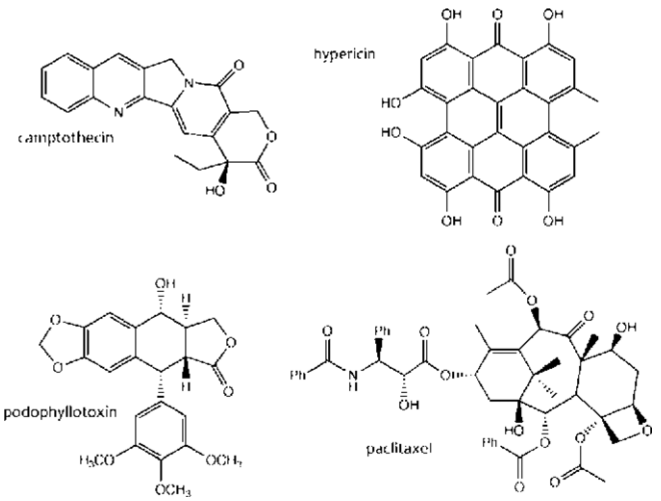

Figure 1. Some important natural products/toxins that are now known to be synthesized by endophytic fungi. Paclitaxel, podophyllotoxin and camptothecin are antitumor agents; hypericin is one component of St. John's Wort, an herb used for mild depression.

Considering their importance, their variety, and their complex role in biology, what then is the Chemist's Perspective on toxic plants? The Chemist's Perspective is very broad: there is significant overlap between the chemical viewpoint and toxicology, as well as pharmacology, pharmacognosy, medicinal chemistry, chemical synthesis, biosynthesis, ecology and alternative medicine. Plants are a rich source of useful materials and interesting scientific investigations. As there are many excellent resources on the toxicology of plants that consider the molecular action of particular molecules isolated from toxic plants [18–20], here I take a different approach. To provide the Chemist's Perspective, or at least one chemist's perspective, I take a more holistic look at the natural products found in toxic plants, and illustrate the connections between chemistry and other scientific disciplines.

Chemosystematics

Systematics is the science that attempts to reconstruct the evolutionary history of life, with the results presented in the form of a phylogeny or "tree of life". Most authorities place taxonomy, which specifically addresses classification issues, within the field of systematics, although not all agree [21]. In any case, humans have been keen observers of plant characteristics and utility throughout the full history of our species; we are all taxonomists whether we are conscious of it or not. Timothy Johns of McGill University makes a compelling argument that humans and plants coevolved in a process in which some plants

were accepted as food, while others were found to be of medicinal value or outright deadly [22]. While the human brain gradually developed an increased capacity for observation and classification, sensory systems such as taste and olfaction, the liver's ability to detoxify an increasing range of xenobiotics and other physiological traits all evolved in a coordinated fashion. At some point, the human species was able to domesticate selected plants, in other words to select and manipulate plants for less toxicity. The invention of cooking, including the possible addition of acid or base, was another innovation to further detoxify plants by chemical and physical means.

The presence of specific chemical entities in plants, and their uneven distribution across the plant kingdom, did not escape early chemists. Morphine was isolated in 1805, long before its structure was correctly described in 1925 [23, 24]. During the 19th century, an increasing number of pure natural products were isolated, although, as with morphine, their structures were not known until much later (Tab. 2, Fig. 2). Structural studies had to wait for organic

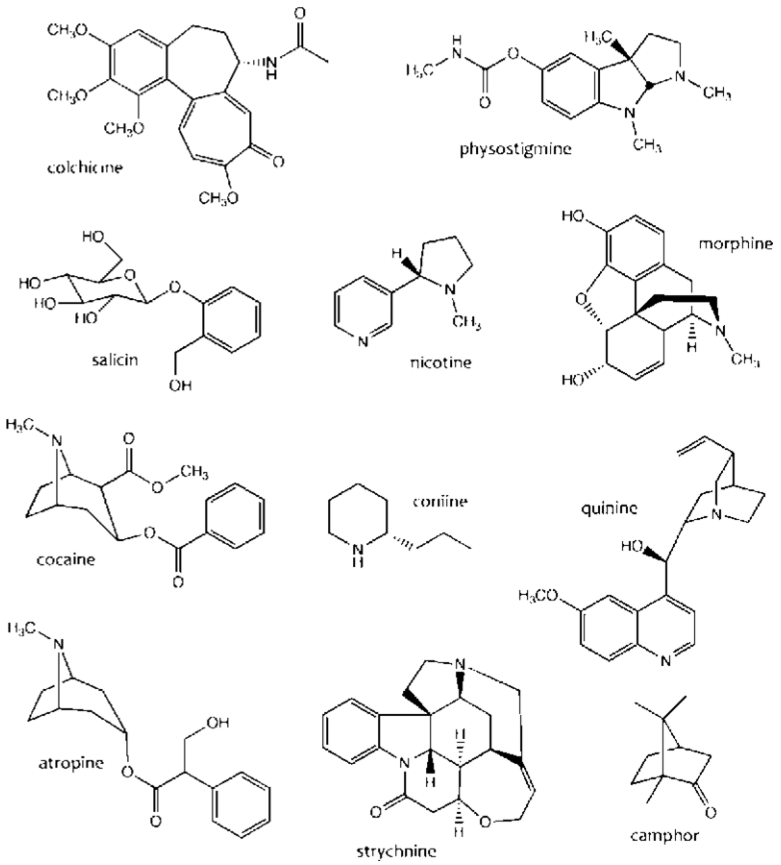

Figure 2. Natural products listed in Table 2.

Table 2. Early milestones in botanically derived toxins and medicinals

Compound	Botanical source	Category	Isolation	Correct structure	Synthesis
Morphine	*Papaver somniferum* (Papaveraceae)	alkaloid	1805 [23]	1925 [152]	1952 [153]
Strychnine	*Strychnos nux-vomica* (Loganiaceae)	alkaloid	1818 [154]	1946 [155]	1954 [156]
Quinine	*Cinchona* spp. (Rubiaceae)	alkaloid	1820 [157]	1918 [158]	1944 [159]
Colchicine	*Colchicum autumnale* (Liliaceae)	alkaloid	1820 [160]	1952 [161]	1961 [162]
Coniine	*Conium maculatum* (Apiaceae)	alkaloid	1826 [163]	1881 [164]	1889 [165]
Nicotine	*Nicotiana tabacum* (Solanaceae)	alkaloid	1828 [166]	1893 [167]	1904 [168]
Salicylic acid/salicin	*Salix* spp. (Salicaceae)	phenol	1830 [169]	1838 (salicin) [170]	1860 (salicylic acid) [171]; 1879 (salicin) [172]
Hyoscyamine/atropine	*Atropa belladonna* (Solanaceae)	alkaloid	1833 (atropine) [173]	1883 (atropine) [174]	1901 (atropine) [175]
Cocaine	*Erythroxylum coca* (Erythroxylaceae)	alkaloid	1860 [176]	1898 [177]	1898 [177]
Physostigmine	*Physostigma venenosum* (Fabaceae)	alkaloid	1864 [178]	1935 [59]	1935 [59]
Podophyllotoxin	*Podophyllum peltatum* (Berberidaceae)	lignan	1880 [179]	1951 [180]	1962 [181]
Camphor	*Cinnamomum camphora* (Lauraceae)	terpene	antiquity	1903 [182]	1903 [182]

Compounds that were described early are dominated by alkaloids, as they often readily crystallized as salts in pure form. An exception is coniine, whose free base is a liquid and which was the first alkaloid synthesized. The synthesis of several of these compounds was the subject of some dispute and drama. For quinine, see [57, 58]; for physostigmine, see [60]. In a number of cases, the synthesis of the compound was also the proof of structure.

chemistry to mature, as a modern understanding of bonding and structure did not coalesce until the latter half of that century. Significant improvements in laboratory methods and technology were also needed before structures could be confirmed. (The development of separation science, using paper chromatography, is one example.) Nevertheless, broad chemical classification of natural products and at least a partial description of properties was possible. Scientists began to realize that certain classes of chemicals were widely distributed, and others narrowly, in the plant kingdom as understood at that time. De Candolle published perhaps the earliest description of this sort, but well before any significant chemical understanding was available (1804) [25]. More chemically enlightened botanical surveys were not available until about 100 years later, with the work of Abbott (in 1896) [26] and Greshoff (in 1909) [27]. The latter coined the phrase "comparative phytochemistry" which was described as "the knowledge of the connection between the natural relationship of plants and their chemical composition". This definition set the stage for further development (see [28] for an excellent history of the field). Since that time, information about the chemical constituents of plants and their distribution continued to accumulate at ever increasing rates. The state of the art by the 1980s is exemplified by Harborne and Turner's "Plant Chemosystematics" [29]. Chapter 12, "Application of Chemistry at the Familial Level", describes surveys of various compound classes and maps them onto plant phylogenies popular at the time. One of the more broadly accepted phylogenies originated with Dahlgren [30]. Figure 3 shows his arrangement of plant superorders with the distribution of benzylisoquinoline alkaloids (BIAs) superimposed. This

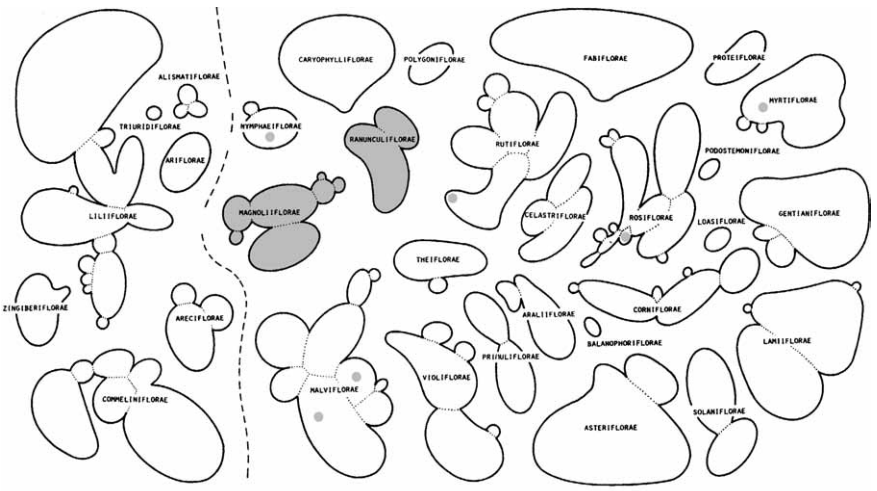

Figure 3. A Dahlgrenogram showing the distribution of benzylisoquinoline alkaloids (BIAs), as understood in 1980. Superorders known to produce BIAs are shown in gray. Adapted from Dahlgren [30] with permission of the publisher, Wiley-Blackwell.

alkaloid family consists of about 2500 different structures and includes important substances such as morphine, codeine and tubocurarine.

The entire landscape of chemosystematics changed dramatically, however, in the 1980s with the biotechnology revolution. The ease and availability of DNA sequencing, cloning and particularly the polymerase chain reaction (PCR), changed the very nature of what was possible in two broad ways. First, plant systematicists had entirely new information with which to construct a phylogeny of plant families, as extensive sequences of chloroplast and mitochondrial DNA became available. These molecular phylogenies turned out to be broadly similar to earlier phylogenies worked out based upon morphological details, reproductive strategies and chemical markers. However, a number of classifications were changed, particularly at the family level. By 1998, enough data was available to assemble a truly modern phylogeny of flowering plants, and in 2003 a significant update was issued [31, 32]. Consider the following simple example which illustrates the significant changes that occurred in thinking about the relationships between plants. For decades, at some point in their science education, young students have typically learned that plants can be divided into two main groups, the monocots and dicots (strictly, monocotyledons and dicotyledons). This grouping followed the scientific thinking common up until the late 1980s. Indeed, readers may remember learning that monocots have parallel leaf veination, while dicots have a network of veins, or that monocots have flower parts in multiples of three's. With the advent of modern molecular phylogenetic methods, we now know that plants placed in the monocots are indeed truly related to each other, because the molecular data coincides with morphological and other data. Under scrutiny, however, the dicots have not held up as a group, although a large portion of them are indeed related and are now known as the eudicots, or true dicots [21, 33].

The second change made possible by biotechnology was that these tools altered the way chemists could investigate the biosynthetic pathways leading to natural products. Early approaches to biosynthesis studies involved detailed tracking of molecular skeletons as they were gradually modified by the plant. These approaches typically involved experiments in which isotopically labeled simple precursor molecules were made available to the plant, such as acetate ion labeled with ^{13}C at either carbon. Later, the natural products were isolated, and the location of the isotopic labels investigated spectroscopically. (Herbert's text [34] exemplifies this approach; but even earlier approaches employed radioactive labels, with subsequent laborious chemical degradation.) The steps employed by the plant to construct the molecule could then be deduced. (Deducing the pathway was much easier if mutant strains could be found or created that lacked certain enzymes along the biosynthetic route. These individuals would accumulate the intermediate ahead of the missing enzyme.) With the new biotechnology tools, one could investigate these processes much more thoroughly and quickly by studying the enzymes that carried out the transformations, rather than the products of those transformations. For example, once a particular enzyme had been identified as carrying

out a reaction of interest, the DNA sequence coding for that enzyme could be used to query databases for other species that possessed a similar or closely related enzyme. As genomic sequence data became available for more and more organisms, comparative studies over large numbers of plant species became possible. Alternatively, the DNA sequence could be used to create a probe for the mRNA coding for a particular enzyme in individual plants. A few examples of this strategy in action are discussed here, but for a full perspective of how biotechnology has changed the study of plants, please see the section on systems toxicology later in this chapter.

Phylogenies constructed prior to the widespread availability of sequence information had always reflected some curiosities in the distribution of natural products. It seemed unlikely that some complex molecular skeletons, which required a significant resource investment by the plants, would be isolated from apparently unrelated families. However, it was difficult to determine if these observations were real, or were due to mistakes in construction of the phylogenies, or perhaps due to incomplete information because an insufficient number of plant species had been studied in detail. As modern phylogenies became available, the occurrence of natural product families and the presence of particular molecular skeletons were mapped onto the new phylogenies. The results were fascinating. In certain plant families for which detailed data were available, it was clear that the presence of particular molecular skeletons was not evenly distributed. A good illustration comes from the laboratory of Michael Wink at University of Heidelberg [35]. Wink and coworkers examined the distribution of quinolizidine alkaloids and non-protein amino acids, two toxin classes common in the Fabaceae family (the legume or bean family). Figure 4 shows that the distribution of these two groups is not even across a number of representative species in this family, which contains about 18 000 species. This figure reveals another interesting finding, namely that species that contain quinolizidine alkaloids typically do not contain non-protein amino acids and *vice versa*; that is, the two categories do not overlap. Apparently, certain lineages have committed to the use of one toxin rather than the other, and resources are not wasted synthesizing both compound classes.

This uneven distribution of compounds could be explained in a number of ways. One could argue that at least some aspects of the distribution suffer from artifacts, such as chemical analyses that are too crude to detect low levels of compounds, or analyses that are not sufficiently selective and give false positives. Another possibility is that the compounds are not actually synthesized by the plants, but rather by endophytic fungi as previously discussed, and hence a plant phylogeny is irrelevant. However, if one assumes that these potential artifacts are fairly rare and that true errors are randomly distributed, then the observed distribution still begs for an explanation. Three explanations are consistent with a modern understanding of the mechanisms of evolution: the same enzymatic capacities have arisen several times independently (convergent evolution), or the genes for synthesizing both compound categories are present in

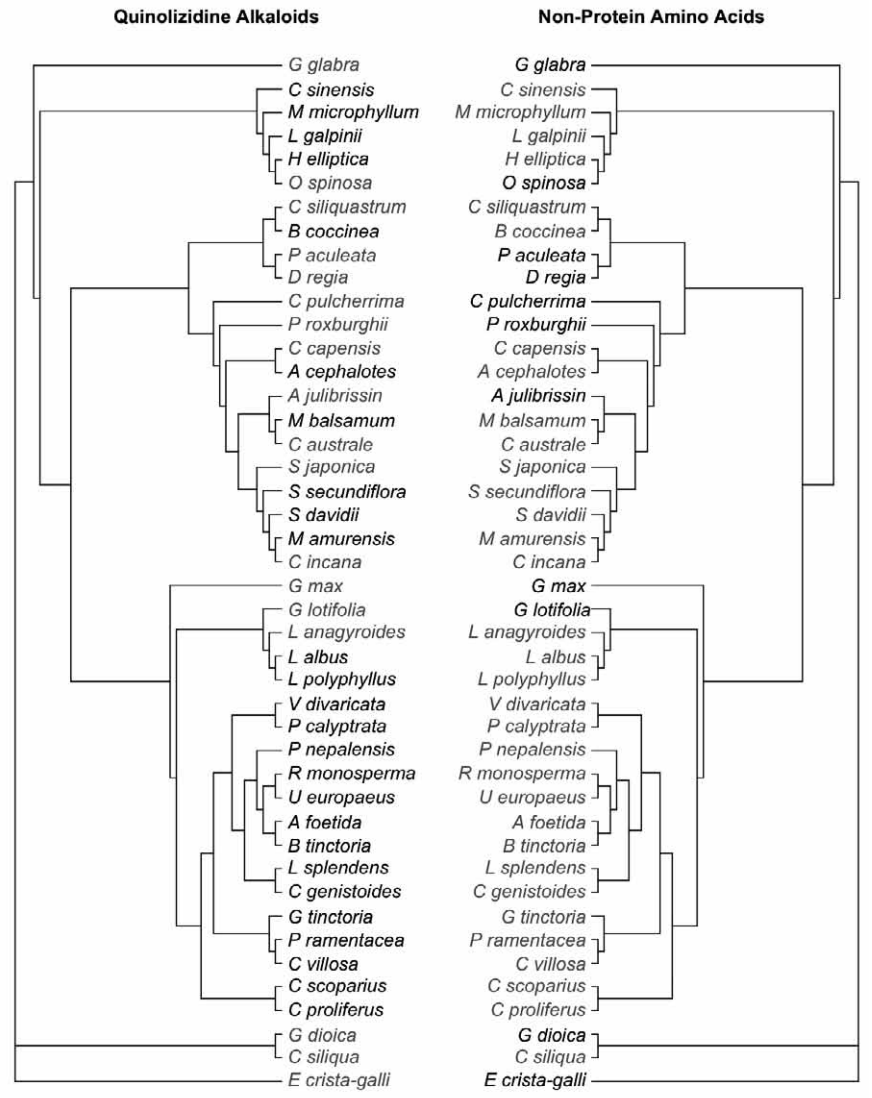

Figure 4. The distribution of quinolizidine alkaloids and non-protein amino acids in the Fabaceae. Bold names are species which contain the compounds, grey names are species in which the compounds are absent. After Wink [36]; based upon data deposited at the European Molecular Biology Laboratory.

all members of the Fabaceae but are turned off in certain taxa, or the genes have been lost in some taxa. Wink's work does not directly address this distinction, however, as his phylogenies are constructed using the sequence of the *rbc*L chloroplast gene, which reflects the overall evolution of the species, not the actual enzymes synthesizing the compounds studied [36].

Workers in Peter Fachinni's laboratory at the University of Calgary have pushed the analysis a step deeper in their study of the distribution of the BIAs mentioned previously [37]. This group of compounds are found primarily in the order Ranunculales but examples are known from other orders (see Fig. 5 for typical structures). A wide range of species were sampled for (*S*)-norcoclaurine synthase activity, the enzyme ultimately responsible for the synthesis of all BIAs. Molecular phylogenies were constructed using the gene sequence for the synthase along with the sequences for several other enzymes unique to selected BIA subpathways. These data were compared with the distribution of the alkaloids mapped onto a phylogeny constructed using chloroplast genes. The results strongly suggest that the genes for the biosynthesis of BIAs are present in a much wider range of plants than just those species from which the alkaloids have been isolated. Hence, the hypothesis that genes for the synthesis of natural products are widespread but turned off in various taxa appears to be strongly supported. In the case of the BIAs, the data suggest that the necessary genes originated prior to the origin of the eudicots; in other words, quite early in the evolution of plants. These results (e.g., Fig. 4) can be compared to

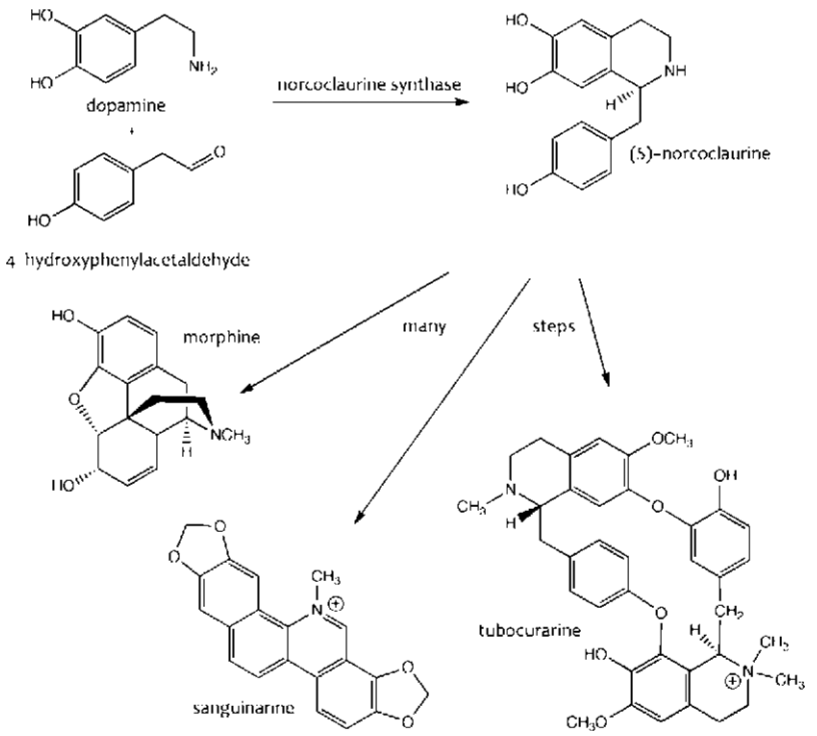

Figure 5. The synthesis of all benzylisoquinoline alkaloids (BIAs) begins with the action of norcoclaurine synthase, and leads to a wide variety of structural skeletons. Only a few examples are shown here.

the Dahlgrenogram presented in Figure 3; clearly, modern analyses differ significantly in their very nature and certainly in their detail.

In contrast, an alternative scenario appears to operate in the case of the pyrrolizidine alkaloids (PAs), a diverse group of toxic substances. PAs are found in rather distantly related plant groups, including eudicots (families Asteraceae and Fabaceae) and monocots (family Orchidaceae). PAs have been implicated in cases of poisoning with herbal medicines (due to contamination [38–41]) and cause liver failure in livestock grazing on *Senecio* species (the ragworts and groundsels, family Asteraceae). PAs are activated in the liver, producing a metabolite that reacts with DNA to give a tumorigenic adduct [42, 43]. An early step in the synthesis of PAs is the conversion of the diamine putrescine to homospermidine, by transferring a C_4NH_2 chain from spermidine. This reaction is carried out by homospermidine synthase (HSS). Elaboration of homospermidine leads eventually to the necine base characteristic of PAs; additional steps add the diester-containing ring (Fig. 6). Work in the laboratories of Dietrich Ober and Thomas Hartmann at the Technical University of Braunschweig has revealed that HSS has likely arisen at least four separate times over evolutionary history [44]. This conclusion was reached by the analysis of amino acid sequences and genomic DNA of a number of species. It appears that the gene for a different enzyme, deoxyhypusine synthase, was duplicated, and the second copy underwent additional evolution

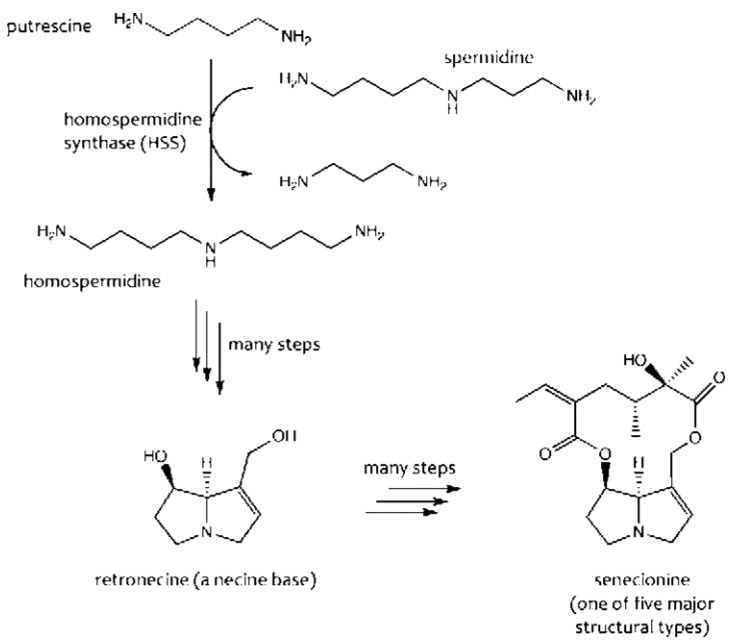

Figure 6. The biosynthesis of pyrrolizidine alkaloids begins with homospermidine synthase, and leads to a diverse array of toxic alkaloids.

to become HSS [45]. Deoxyhypusine synthase also transfers a C_4NH_2 chain from spermidine, but in this case the acceptor is a lysine side chain of a transcription factor. This is an example of convergent evolution by change of function after duplication. Other examples in which a no-longer-needed copy of a gene evolves to have a new function and eventually different product specificities and expression patterns are known from the terpene and flavonoid pathways [46].

In contrast to these more complex scenarios, there are some compound families which are narrowly distributed, suggesting that their biosynthetic pathways originated more recently. Perhaps the best example is that of the betalain pigments, which are found exclusively in the order Caryophyllales and serve as a reliable marker for this order [47, 48].

These examples demonstrate that there is a great deal to be learned from the distribution of natural products in light of modern molecular phylogenies. Unfortunately, the hope of systematicists that natural products would serve as simple taxonomic characters has proven to be too good to be true. They certainly can serve as useful markers, but a 'present/not present' interpretation is clearly a too-simple approach. The reality is that the study of natural products and the enzymes that produce them will enlighten systematics and the mechanisms of plant evolution greatly, but considerable investment will be needed to work out the details [28].

Chemical synthesis and structural diversity

The laboratory synthesis of natural toxins and medicinal substances has always been of great importance; it is the basis for the modern pharmaceutical industry. Typically, once the structure of a natural product has been described, there are always chemists ready to undertake its synthesis, although in one celebrated instance, these steps were reversed. In 1856, William Perkin undertook the synthesis of the critically important antimalarial quinine starting from aniline, even though he did not know the structure of quinine, which was not described until 1918. While this experiment was extraordinarily naïve in retrospect, Perkin did discover the compound mauveine, which launched the entire synthetic dye industry [49]. The synthesis of quinine was ultimately a much more difficult task, as it was not until 1944 that the synthesis was achieved (but not all agree about this, as described below).

"*It is well worth looking at the proposition that chemical synthesis is an art form, needing no justification because it permits self-expression in its creators and produces aesthetic pleasure in those who examine its products.*"

J.W. Cornforth [50]

There are a number of reasons that chemists pursue synthesis. There are those who view the synthesis of complex molecules as a Mount Everest to be climb-

ed or a chess game to be won [51–53]. Since the resulting synthetic schemes are generally long and complex, it is hard to argue that the process will lead to the preparation of large quantities of material for clinical or commercial use. Along the way, however, new reactions may be invented, which might be more efficient with regard to building up the skeleton or controlling stereochemistry, or which may be more environmentally friendly. The preparation of simpler analogs, often undertaken as an intermediate step in the synthesis of a more complex target compound, may lead to compounds that retain biological activity, which in turn gives insight into the pharmacophore and suggests other compounds to prepare.

Occasionally, the synthesis of a reported compound leads to the discovery of errors in the original structure, and to their correction [54]. And controversies arise from time to time. The synthesis of quinine, reported in 1944 [55], has been called into question with the publication of a stereoselective synthesis in 2001 [56]. The interesting story about who made what compound and when they made it has been analyzed in detail by Seeman [57] and reveals quite a bit about the science of total synthesis (see also the account by Kaufman and Rúveda [58]). Similarly, the synthesis of physostigmine, an ordeal poison used in traditional jurisprudence by certain African cultural groups, was achieved by African-American chemist Percy Julian working at a small college in rural America in 1935 [59]. This was an extraordinary achievement at the time, and all the more interesting because Julian completed the synthesis ahead of the very accomplished research group of Sir Robert Robinson at Oxford. In addition, Julian showed that Robinson had been wrong in some of his earlier publications. Addison Ault has provided a concise description of how Julian did it, and how Robinson was misled [60].

One of the most interesting debates in the field of synthesis is the issue of what molecules to make, and how to go about making them. A traditional approach favored by those who see synthesis as a chess game is to choose biologically active molecules that have high degrees of complexity or which have carbon skeletons that have not previously been made. (Funding is much easier to obtain for molecules which have biological activity of potential medical interest.) The chosen molecules are then synthesized by some combination of known reactions, or reactions which must be invented, often in very long sequences. This approach does not correspond to the needs of the pharmaceutical industry, where simpler molecules with high biological activity and good therapeutic profiles are mandatory, and shorter syntheses are critical. Consequently, a great deal of thought and strategy has gone into inventing alternative methods for choosing and making molecules, with the goal of minimizing the time necessary for discovering novel (i.e., patentable) active compounds which are easily made at reasonable cost.

Over the long haul Nature has provided a large fraction of our useful molecules, and the natural world continues to be a source of inspiration and ideas as discussed in the first section. However, it can be argued that most molecules with high biological activity, and which are present in modest quantities in

their natural sources, were easy to discover and have already been exploited; hence, different approaches are needed to develop new drug candidates. One approach to thinking about this issue is to recognize that the potential structural diversity of small to medium-sized organic molecules is enormous, and can be described in a number of ways, some of which may lead us to new ideas. These descriptors include such things as connectivity, lipophilicity, topology, the functional groups present, chirality, flexibility and so forth. Collectively, these and other descriptors have been called the "chemical space" in which molecules exist [61]. The challenge in finding new useful molecules is then twofold: first, to describe this chemical space accurately, and second, to map the chemical space onto the corresponding biological-activity space in such a way that useful activity is found more quickly. One could argue that this is exactly what Nature has done through the process of evolution: sampling a wide swath of chemical space in search of a hit in biological-activity space. Biologically active natural products have been described as "evolutionarily selected", "prevalidated" or "privileged" by various authors.

This notion of chemical space and characterizing it is not really new; it is the basis for much of medicinal chemistry and rational drug design using quantitative structure-activity relationships (QSAR). However, the growth of publicly available databases has facilitated new approaches. Lipkus and colleagues at the Chemical Abstracts Service have analyzed their database of more than 24 million organic compounds described in the literature to measure their structural diversity [62]. Their results demonstrate that the number of known skeletons is actually quite limited and that most compounds are derivatives of these known skeletons, suggesting that true structural diversity is low (Bohacek has estimated the number of possible structures at 10^{60} [63]). Feher and Schmidt [64] have conducted a statistical analysis of the similarity of natural products, drugs on the market, and molecules made by combinatorial chemistry. (Combinatorial chemistry is the rapid, high-throughput assembly of modest size molecules from a set of building blocks in a somewhat randomly selected fashion. The result is a set – library – of molecules from which the active ones can hopefully be fished out by an appropriate assay.) They found that the chemical space explored by combinatorial chemistry appears to be significantly limited by the reactions typically employed in combinatorial work. In contrast, drugs in use and natural products cover a much greater volume of chemical space. Waldmann and colleagues at the Max Planck Institute of Molecular Physiology in Germany have carried out a similar structural classification of known natural products but have gone beyond mere description and used the results to design new drugs [65]. They have also reviewed recent approaches to describing chemical space [66]. Finally, researchers at Uppsala University and AstraZeneca have described ChemGPS-NP, whose name emphasizes the need to navigate within this chemical space [67]. All these studies reach the same general conclusion, namely that the information and diversity in natural product structures is underutilized relative to the full potential of chemical space.

As descriptions of chemical space have been refined, the questions of how this space relates to biological-activity space and which compounds to make has developed simultaneously [61, 68–71]. New approaches have been developed that consider, in principle, all (or at least more) of the possible chemical space, and which try to address a broad region of biological-activity space as well. These explorations have led to a number of interesting drug discovery strategies.

Foremost among these are investigations in which the principles and concepts of combinatorial chemistry are merged with the notion of using natural products directly as scaffolds, or as the inspiration for scaffolds [72]. The basic procedure is to begin with a natural product or perhaps a simplified version, and attach it to a resin for subsequent modifications by solid-phase synthesis. One then uses the existing functional groups to modify the structure, in effect adding a wide variety of "side chains" at several different sites. An alternative approach is to introduce the same building blocks, but with differing chirality. An example based upon the toxin galanthamine is shown in Figure 7 (the spelling in some publications is galantamine). Galanthamine is a selective and competitive acetylcholinesterase inhibitor found in the family Amarylidaceae [73], such as the bulb of the common daffodil (*Narcissus pseudonarcissus*). Developed from indigenous knowledge, it has recently become available for the treatment of Alzheimer's disease [74]. Shair and colleagues at Harvard have developed a library of compounds that are based upon a modified galan-

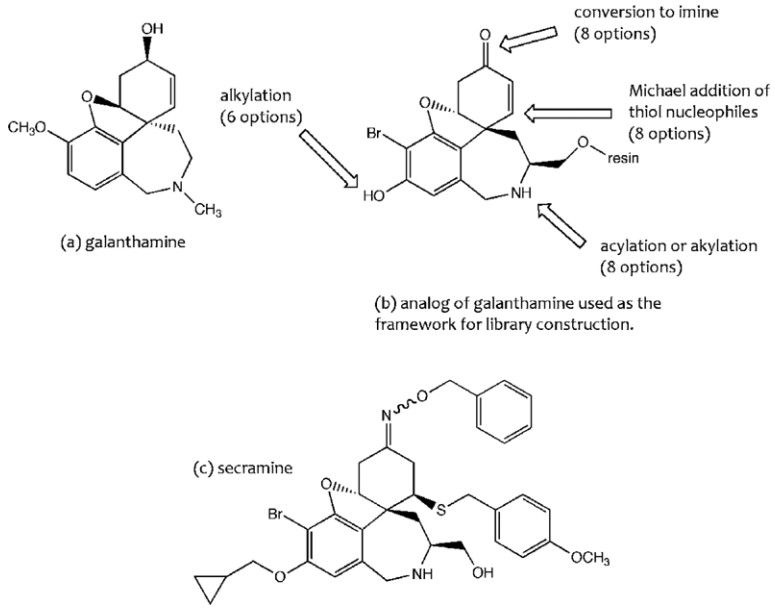

Figure 7. (a) Galanthamine; (b) strategy for construction of a library based upon galanthamine; (c) secramine, a structure isolated from the library with completely different biological activity.

thamine structure [75]. Beginning with an analog constructed on a solid resin, a variety of side chains were introduced in all possible combinations using the instrinsic reactivity of the analog's functional groups, leading to a library of 2527 different molecules (about 85% of the theoretical number). Screening of this library lead to the isolation of secramine (Fig. 7c), which is an inhibitor of protein trafficking, a biological activity completely unrelated to that of galanthamine. Many other examples employing a similar combinatorial approach have been reported [76, 77] and there is great promise for discovery of new structures with new activities.

Another approach to generating structural diversity takes advantage of the fact that many natural products are present as glycosides, that is, in combination (conjugation) with sugars. The function of these sugars is to increase the solubility of the often non-polar molecule (the aglycone) in the aqueous environment of the cell. The nature of these sugars, as well as their presence or absence, often has a large effect on their biological activity [78]. A typical and important example is that of digitoxin, derived from the Foxglove plant (*Digitalis purpurea,* Plantaginaceae), the subject of one of the earliest known clinical trials [79] (Fig. 8). Thorson and colleagues at the University of Wisconsin have developed several means of generating structural diversity by adding non-natural sugars to the aglycones, as well as methods for randomizing the sugars present using glycosyltransferases which are able to accept a variety of sugars as substrates (so-called promiscuous enzymes). Applying this approach to digitoxin, they created a library of 78 analogs by replacing the triose of digitoxin with a variety of monosaccharides, and varying the stereochemistry at the point of attachment [80]. The normal activity of digitoxin is to increase the force of heart-muscle contraction by inhibiting Na^+/K^+ ATPase activity. It also exhibits modest but non-specific cytotoxic effects on cancer cell lines. Bioassay of this library against various cancer cell lines led to the discovery of members with much more potent or selective cytotoxicity (but not both). Thorson has also created a library of 58 glycosides of the tubulin polymerization inhibitor colchicine (Fig. 2), a molecule that does not normally exist as a glycoside [81]. Once again, some members of this library exhibited greater potency or selectivity, and two members stabilized the structure of tubulin, the

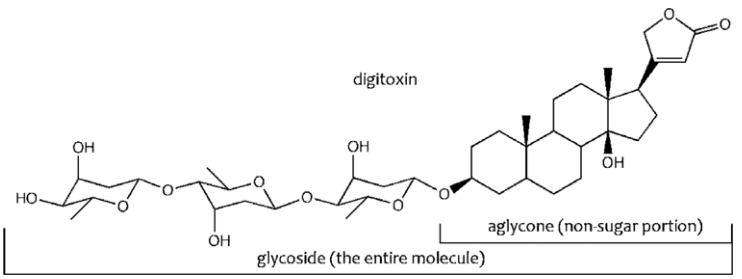

Figure 8. The structure of digitoxin, a glycoside that acts to increase the force of heart contraction.

opposite effect of colchicine. Digitoxin and colchicine are compounds from toxic plants, but the Thorson group has demonstrated the broad applicability of this approach with medicinal compounds from bacteria and fungi, and has shown that the enzymes involved can be engineered to great advantage [82–85].

"*… I see no reason why we should not welcome enzymes and microbes as friends and colleagues. Since they work for even less than graduate students, perhaps we should at least acknowledge them…*"

J.W. Cornforth [50]

In addition to strategies designed to create a greater diversity of chemical structures, scientists have sought to carry out the syntheses of naturally occurring compounds in more efficient ways, an approach that has benefited greatly from the developments in biotechnology described earlier [86]. One approach is to abandon traditional synthesis and develop cell cultures and other systems that produce the natural products of interest.

Several excellent examples exist; perhaps the most important is that of paclitaxel (trade name Taxol). This compound was one of several to be developed principally by Wall and Wani at Research Triangle Park [87]. Paclitaxel was first isolated from a thin layer of inner bark of the pacific yew tree (*Taxus brevifolia*, Taxaceae) in 1971. Its mode of action was unique at the time (tubulin stabilization [88]) and it was eventually marketed by Bristol-Meyers Squibb for the treatment of ovarian, breast and lung cancers. As its efficacy became apparent, problems quickly arose with the supply of the drug. Isolation from the tree was clearly untenable as it would create a considerable environmental disaster if pursued (one mature tree would produce about one dose) [89]. Subsequently, related species were found that produced paclitaxel or related structures, and these provided the supply necessary for clinical use. Even so, the compound is still quite expensive, about $ 300 000 per kilogram. Consequently, much effort has gone into studying the biosynthetic pathways leading to paclitaxel in the hopes of harnessing the enzymes. In addition, many investigators have worked on developing plant cell culture methods for the production of paclitaxel or a related molecule that can be converted to it in a cost-effective manner [90, 91]. Phyton Corporation produces paclitaxel in a 75 000-L fermentation/cell culture system. Current research is aimed at optimizing the cell culture conditions for initial growth, after which the cells are transferred to a different media that enhances the production of paclitaxel. The discovery that paclitaxel is apparently synthesized by an endophytic fungus (detailed earlier) has both complicated and simplified efforts. The important antimalarial artemisinin from *Artemisia annua* (Asteraceae) is currently going through much the same development cycle as paclitaxel [92].

A strategy that is both potentially very efficient and amenable to generating structural diversity is to genetically engineer microorganisms to carry out the syntheses [93]. In this so-called combinatorial biosynthesis, genes from a plant (possibly more than one) are moved into a different organism such as a bac-

terium or a yeast, and in combination with the native genes of that organism, they may be coaxed into synthesizing a desired product. The hope is that the heterologous system may be more practical in terms of the ease of culture and the production efficiency (Kayser and colleagues have reviewed a number of such investigations [94]). In addition to making the synthesis more efficient, such systems may be engineered to rearrange the order of genes and even combine genes from different organisms to produce novel structures, which may have novel mechanisms of action.

A good illustration of the first approach involves the BIAs discussed earlier. These compounds are synthesized in plants beginning with the action of norcoclaurine synthase, followed by a wide variety of additional enzymes depending upon the carbon skeleton found in a particular species (Fig. 5). Minami and Sato in Japan developed a two-organism, one-culture method for the efficient preparation of BIAs. These workers first prepared and cultured a transgenic *Escherichia coli* line with the plant genes for the synthesis of (*S*)-reticuline, a key branch point in the pathways leading to diverse BIAs. After a period of time, they added to the growing bacterial culture a transgenic *Saccharomyces cerevisiae* that contained additional genes for the transformation of reticuline into magnoflorine. In a second experiment, the added transgenic yeast contained the genes for the synthesis of scoulerine. These co-culture systems, containing transgenic plant genes carried in two different organisms, and supplemented by bacterial enzymes, were able to produce good quantities of structurally diverse alkaloids (Fig. 9) [95]. Both magnoflorine and scoulerine are of medicinal interest, but the success of this method opens the door to the synthesis of BIAs of even greater medical importance. A similar investigation has been reported by Hawkins and Smolke at the California

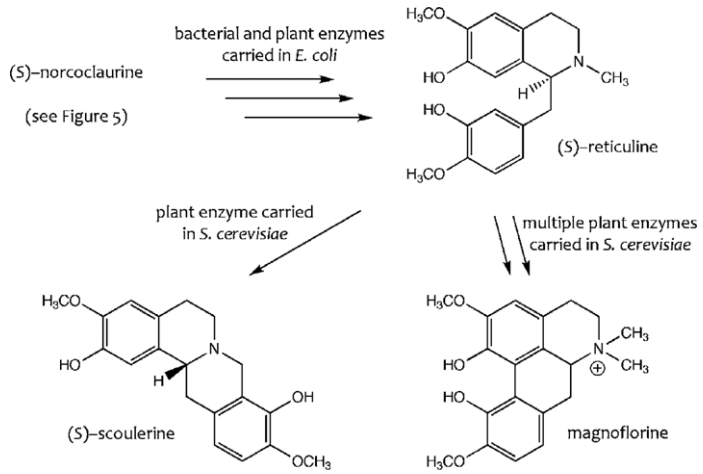

Figure 9. The Minami and Sato co-culture system for the preparation of benzylisoquinoline alkaloids (BIAs).

Institute of Technology using yeast cells containing plant and human enzymes [96]. This system was shown to synthesize a morphine precursor, in addition to sanguinarine/berberine skeletons.

A second and particularly ambitious example is the attempt by Verpoorte and colleagues to produce *Vinca* (*Catharanthus*) alkaloids in heterologous systems (Fig. 10) [97]. Vincristine and vinblastine are very important antineoplastic compounds and among the most structurally complex plant natural products known. They are produced in plants at extremely low levels, and cell culture methods have not been successful. Hence, there is great interest in a biotechnological solution. Unfortunately, the biosynthetic pathway involves at least 32 genes and 35 intermediates, along with 7 subcellular compartments (which is consistent with the structural complexity of the compounds). While portions of the pathways have been successfully transferred to *E. coli, S. cerevisiae* and *Nicotina tabacum*, efficient expression of the entire biosynthetic apparatus has not yet been achieved. However, McCoy and O'Connor at Harvard University have reported that seedlings and hairy root cultures of *Catharanthus roseus* are able to accept a wide variety of substituted tryptamine precursors and carry them through to compounds late in the biosynthetic sequence [98, 99].

The second broad approach, to generate novel structures by combining and re-ordering genes from several plants, has only recently begun to be explored. Polyketide synthases (PKS) are responsible for the synthesis of a wide range of interesting natural products. These modular enzyme complexes are able to build up a carbon chain from a variety of starting units, add multiple extender units, and modify the resulting structure by various combinations of cyclizations, reductions and dehydrations. Over evolutionary time, the individual genes in these complexes have been duplicated and subsequently modified, creating a set of tools that can accept different substrates, and be used in different orders for different results. In other words, Nature has been employing

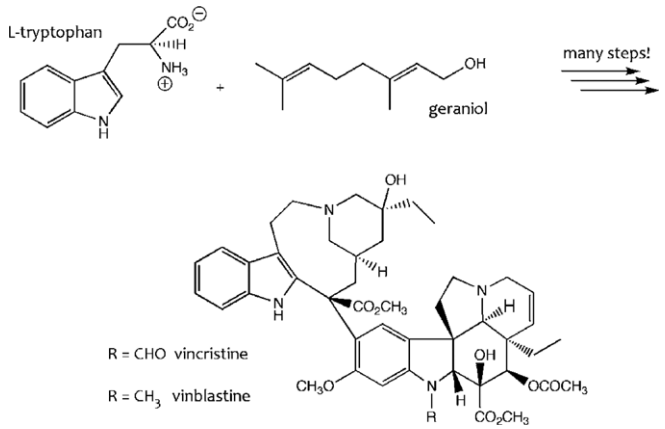

Figure 10. The biosynthesis of the *Vinca* alkaloids vincristine and vinblastine.

a sort of combinatorial biochemistry that humans have only recently recognized and tried to manipulate. The chemistry and genetic engineering of PKS have been exploited for sometime in bacteria [100], but the plant enzymes have only recently been cloned and put to use [101, 102]. By rearranging the order of individual enzymes that carry out the cyclizations and other modifications, new structures can be created. Some PKS are promiscuous as they will accept a variety of starter units not found in Nature, which permits additional structural diversity. Figure 11 illustrates the overall process and structures of a few important plant natural products generated by PKS.

Choosing a target for synthesis has clearly moved well beyond early motivations. The means of synthesis have also changed significantly. The examples described above demonstrate that the field of synthesis remains a very creative and practical endeavor. While Nature has provided numerous useful drugs and toxins, it is clear that creative chemists and molecular biologists will continue to harness the tools that Nature has been using to create even more structural variety and to do so by increasingly efficient means.

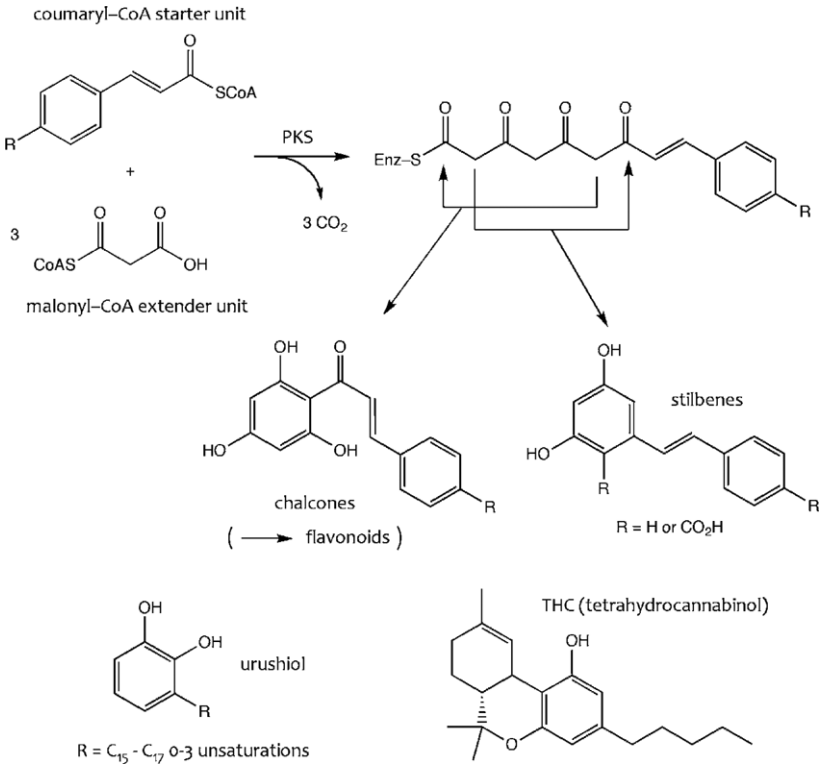

Figure 11. A typical plant biosynthetic pathway leading to polyketide intermediates, which can be cyclized to give a variety of structures. PKS, polyketide synthase. THC (Δ^9-tetrahydrocannabinol) is the active ingredient in marijuana. Urushiols are the active ingredients in poison ivy.

Systems toxicology

One of the most interesting recent developments bridging chemistry and biology is the development of systems biology, which seeks to integrate knowledge of the molecular workings of living organisms across several levels. Crick's "central dogma" is the unifying concept in molecular biology, which describes the flow of information in an organism, from DNA to RNA and finally to proteins [103]. The development of biotechnology led to an understanding of this information flow on a much grander scale within a single organism, and in a comparative fashion between organisms. Beginning with genomics (e.g., the human genome project), and later proteomics and transcriptomics, the available information has exploded in quantity and improved in quality. More recently, the field of metabolomics has made its debut – metabolomics studies the result of the flow of information out of the central dogma as well as its regulation – in other words, the identity and concentrations of all metabolites in an organism. Systems biology is an attempt to use all this information at once to study how the pieces function in an integrated fashion. Figure 12 illustrates the relationship between these concepts.

The systems biology approach can provide a great deal of information about an organism under normal conditions, but the greatest insight is derived by comparing this reference state to some sort of perturbed or stressed state. For instance, one might study the metabolism of carbohydrates by comparing growth under normal conditions to one in which a particular substrate is lacking or enhanced [104, 105]. Systems toxicology in particular is the study of organisms stressed by some sort of xenobiotic toxin, and has great potential in the pharmaceutical industry. Applications are being developed that use metabolomics to speed drug development by improving the preclinical screening process, the elucidation of metabolic pathways, and the determination of mechanisms of toxicity [106, 107]. Not surprisingly, there is also enormous interest in using these methods to develop diagnostic biomarkers for a wide variety of disease states, and in some cases molecular changes can be detected long before a disease makes its appearance *via* traditional clinical indications [108]. A good illustration of the strategy and potential of the systems toxicology approach is a study conducted by Nicholson's group at Imperial College London and colleagues at AstraZeneca [109]. These investigators studied the necrosis of liver tissue induced by methapyrilene in rats using a combination of gene expression analysis (transcriptomics), a comprehensive analysis of protein levels (proteomics) and NMR spectroscopy of urine and liver tissue samples (metabolomics). These methods were linked to more traditional histological analysis and revealed complex changes in the molecular systems that react to oxidative stress as well as those responsible for energy usage.

As with all applications of systems biology, one of the key challenges is the management of the flood of data that results from these complex studies. Several recent reviews have discussed the development of knowledge bases in

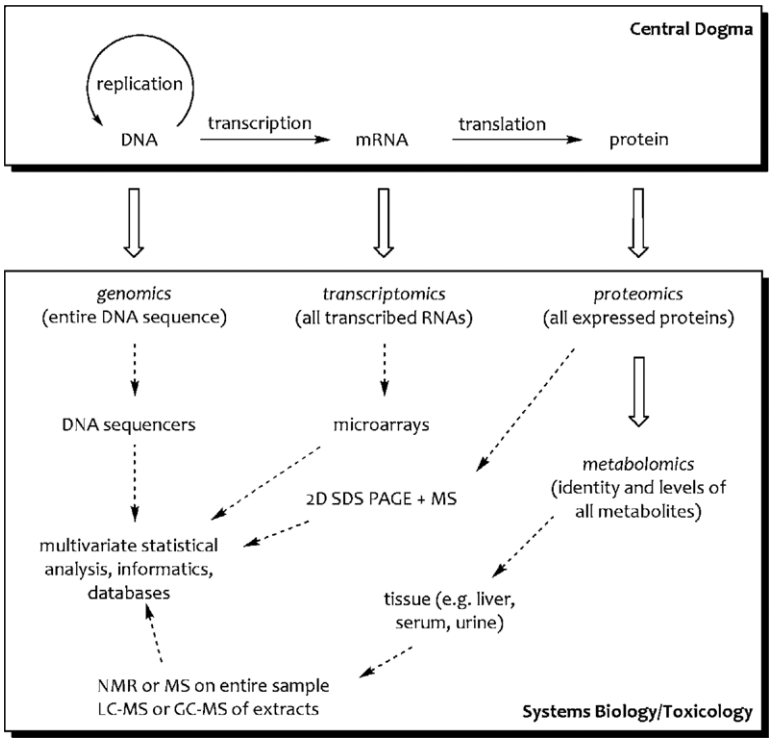

Figure 12. The relationship between the Central Dogma of molecular biology and systems biology concepts. Genomics is the study of the entire DNA sequence of an organism. Transcriptomics is the study of all transcribed mRNA sequences, normally a subset of the entire genome. Proteomics is the study of all expressed (and modified) proteins. Metabolomics is the study of all metabolites and their levels, which result from the action of the proteins through both enzymatic and regulatory activities. Each of these fields has associated techniques, which lead to large data sets that must be analyzed by appropriate statistical methods. MS, mass spectrometry; NMR, nuclear magnetic resonance; LC, liquid chromatography; GC, gas chromatography; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

toxicology that endeavor to integrate the systems approach with more traditional types of toxicological studies [110, 111]. The development of the Chemical Effects in Biological Systems (CEBS) depository is one effort to organize data from a variety of experimental methods in a comprehensive manner so that the results can be mined for insights and compared across laboratories and organisms [112].

Systems toxicological studies of medicinal and toxic plants have only recently appeared in the literature. An area that has been a rich source of research programs are the traditional healing methods of China and India, known as Traditional Chinese Medicine (TCM) and ayurveda, respectively [113]. Both of these traditions are holistic healing paradigms that use a wide variety of individual herbs and especially mixtures of herbs. They are ideal

candidates for the systems approach as the presence of multiple medicinal and toxic substances would be expected to have effects on a wide variety of organ systems. The broad and sometimes subtle effects produced by the phytomedicinal mixtures typical of these traditions are not easily identified or quantified by the usual drug discovery and development processes, which are strongly oriented toward single chemical entities [114]. In addition, the synergistic effects often claimed for herbal mixtures do not fit well with the Western paradigm for healing [115–117]. Several authors have pointed out that the systems approach is an excellent match for understanding the biological effects of these herbal mixtures, one that can provide a bridge between toxicology, molecular pharmacology and ethnopharmacology [118–125].

The most common systems toxicological studies involving plant extracts so far are transcriptomic studies using microarrays to measure changes in gene expression (i.e., mRNA expression levels). An early example demonstrating the power of the approach was the report by Watanabe et al. in 2001 [126] regarding the effect of herb *Ginkgo biloba* (Ginkgoaceae) on the cortex and hippocampus of mice. Ginkgo is an ancient Chinese herb used to treat a variety of cognitive deficits [127]. A number of the individual components of this herb are known to be biologically active as antioxidants and as platelet-activating factor antagonists [128–130]. The study analyzed ~12 000 mRNA transcripts and identified 10 genes that were up-regulated more than 3-fold in mice fed a supplement containing ginkgo. Functional annotation of these genes identified proteins with a role in neurotransmission, cell growth and neuroprotection. Another study involving brain function was designed by Wang et al. [131]. In this work, cerebral ischemia was induced in mice that had been maintained on various dosages of a standardized TCM herbal glycoside recipe, consisting of the compounds baicalein and dioscin (see Fig. 13 for structures of compounds mentioned in this section). These compounds are found in the Chinese herb *Scutellaria baicalensis* (Lamiaceae), which is one of the most important herbs in TCM. Microarray analysis of the hippocampus was coupled with measurements of spatial learning memory, measured by performance in a water maze. These authors found that the herbal treatment led to improved recovery from the ischemia (i.e., a better performance in the maze and a decreased infarct volume). Nine genes were observed to be up- or down-regulated by more than 1.8-fold in the two highest dosages of the herbal treatment. As with the previous study, the roles of these genes could reasonably be associated with improved learning and cognitive function; for instance, expression of the 5-hydroxytryptophan (serotonin) receptor decreased in a dose-dependent manner.

Phytoestrogens are plant compounds that mimic the effect of estrogen in humans. As a type of endocrine disruptor, they are of interest not only from a toxicological perspective, but also as potential treatments for estrogen-sensitive cancers. It is not surprising therefore that a number of researchers have examined the action of phytoestrogens using microarrays. A recent example is the study by several groups in Japan, who examined the effect of various phy-

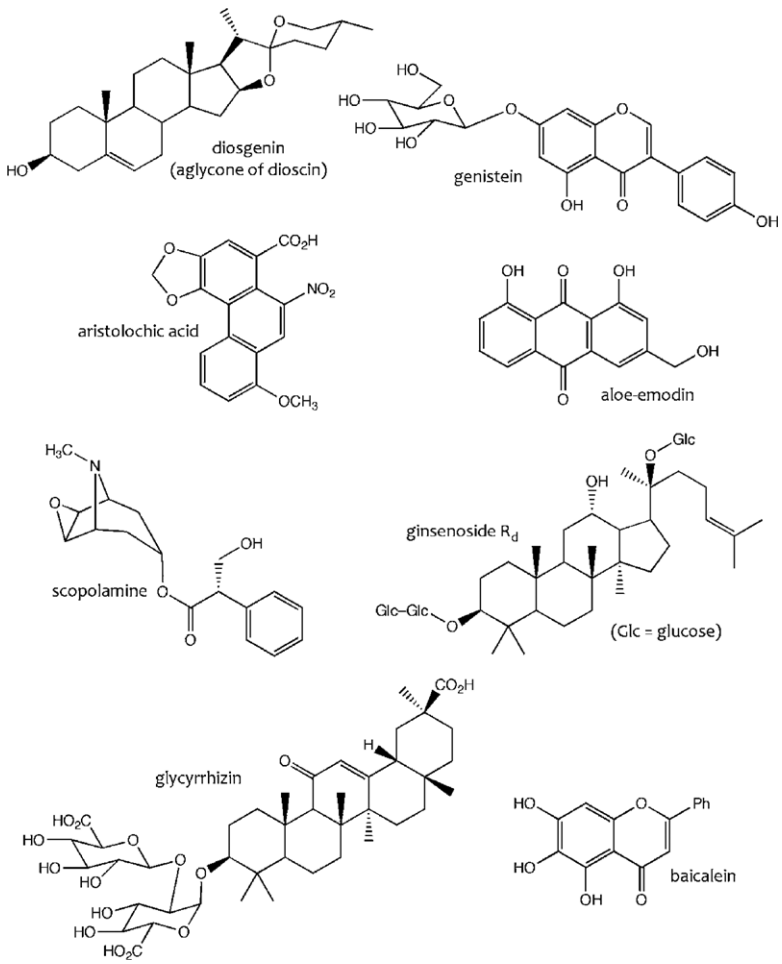

Figure 13. Structures of compounds mentioned in the section on systems toxicology.

toestrogens on human breast cancer cells [132]. Both pure phytochemicals (e.g., the isoflavone genistein) as well as extracts of soy beans (*Glycine max*, Fabaceae) were investigated using a custom microarray composed of genes known to be estrogen responsive. About 20 genes were identified that responded to the phytoestrogens differently than to estrogen. The majority of these genes have a known role in signal transduction in cancer. Another study using breast cancer cells was conducted by Dong and colleagues using extracts of licorice (*Glycyrrhiza glabra*, Fabaceae; licorice is also part of the TCM pharmacopeia) [133]. This extract promotes the growth of cancer cells due to certain components that activate the estrogen receptor. Analysis of the expression profiles allowed the authors to conclude that glycyrrhizin, the main triterpene in licorice, did not induce estrogen-responsive genes or cell proliferation.

Rather, it appears that a number of components acting in concert are responsible for the proliferation. As with the previous study, many genes associated with signaling are affected by the licorice extract. Endocrine disruptors are also known to affect development of the sexual organs *in utero*. Adachi et al. [134] demonstrated that neonatal exposure to genistein had a long-term effect on the expression of the estrogen and androgen receptors in mice testes, even though there were no morphological changes. Naciff and colleagues [135] at Proctor and Gamble also found that genistein had a significant transplacental effect on rat testes, and identified 23 genes that were up- or down-regulated by at least a factor of 1.5. At the highest dosages, 46 genes were significantly affected. The pattern observed for genistein was similar to an estrogen derivative as well as the important industrial toxin bisphenol A, suggesting that these compounds act in a similar fashion.

Several other transcriptomic studies of botanicals have been conducted. Hsieh and colleagues investigated *Scutellaria baicalensis*, mentioned above in a study of cognitive performance, regarding its role as an anti-inflammatory herb, another TCM-use for the plant [136]. Using human embryonic kidney cells, these researchers identified changes in expression of several genes associated with inflammatory and immune responses as the likely mechanism of action of the plant. Some of the same investigators reported a study of the rat hippocampus treated with scopolamine, a substance that induces memory impairment [137]. Key differences between treated and untreated rats involved genes related to the muscarinic receptors, and several others were genes associated with the development of Alzheimer's disease.

Proteomic studies have also been conducted on plants of toxic and medicinal interest, and not surprisingly plants from TCM have been the focus [138]. Two studies have been reported on the genus *Scutellaria*. Ong's group in Singapore studied *S. baicalensis* and its effect on proteins of the mouse liver [139]. At low doses no changes were observed, but at high doses, bile duct damage was observed along with changes in expression of proteins involved in triglyceride-rich particle processing, carbohydrate metabolism, cell signaling and xenobiotic transformation. The same group investigated *S. barbata* and its effect on human colon cancer cell lines [140]. A combined cell-cycle analysis and proteomic investigation revealed that the botanical extract induced cell death, apparently *via* changes in transcription factors and regulation of the cell cycle. Some members of the same group have studied the effect of rhubarb root (*Rheum palmatum*, Polygonaceae), also used in TCM, on human liver cancer cells [141]. Rhubarb contains bioactive anthroquinones such as aloe-emodin that are believed to be responsible for its biological action. The effect of rhubarb seemed to be mediated primarily by up-regulation of proteins involved in the oxidative stress response, which was confirmed by separate biochemical measurements. Other proteins whose expression varied significantly were those responsible for cell-cycle arrest and antimetastasis.

Cheng and colleagues in China studied the TCM material medica ShuangDan Decoction, which is a mixture of two herbs frequently used for

myocardial infarction, angia and coronary heart disease [142]. Proteomic investigation of rat myocardium along with histological and biochemical studies revealed modulation of 23 proteins in ischemic hearts. These proteins generally fell into the categories of energy metabolism, oxidative stress response, and cytoskeleton maintenance. Wink and colleagues [143] investigated the influence of red clover (*Trifolium pratense*, Fabaceae), which contains large amounts of isoflavones, on both gene and protein expression in the liver of ovariectomized rats. They found that plasma lipid levels were differentially affected by the isoflavone treatments, and that genes affecting lipid metabolism and oxidative response were the main protein changes. Interestingly, compared to a limited number of changes in protein expression, there were quite a few changes in gene expression, involving not only lipid metabolism and oxidative responses, but also androgen/estrogen regulation and the metabolism of xenobiotics. Ginseng is another herb used extensively throughout Asia. Lee and colleagues in Korea have examined changes in the proteome of colon cancer cells as a result of treatment with ginsenoside R_d [144]. Significant changes were observed in proteins responsible for apoptosis, DNA replication and repair, protein synthesis and degradation, and mutagenesis. Although not an investigation of an animal model affected by a toxic/medicinal plant, as part of their overall investigation of *Vinca* alkaloids, Verpoorte and colleagues [145] have reported on the proteomics of cell suspension cultures of *Catharanthus roseus*. This study revealed some interesting insights into the biosynthesis of these alkaloids. Among other discoveries, the authors were able to identify two isoforms of strictosidine synthase, one of the key early enzymes in the biosynthetic pathway. This sort of study illustrates how the systems approach can also inform the taxonomic, biosynthetic and applied studies discussed earlier in this chapter.

Metabolomic studies of toxic and medicinal plants are just now beginning to appear in the literature. (There are a fairly large numbers of metabolomic-type studies on medicinal plants aimed at quality control and authentification. These are not discussed here as they do not directly deal with toxicology in organisms treated with the plants.) Chen et al. [146] studied the effect of aristolochic acid on rats. Aristolochic acid is a well-known nephrotoxin found in members of the family Aristolochiaceae. The acid and its derivatives have also been implicated as contaminants in various herbal mixtures, and cause serious problems such as acute renal failure and end-stage renal disease. These authors combined traditional histological examination with liquid chromatography–mass spectrometry (LC-MS) of urine using pattern recognition methods. Comparison of untreated rats with rats receiving pure aristolochic acid as well as a TCM preparation from the plant *Aristolochia manshuriensis* led to the discovery that metabolic pathways involving homocysteine and folate appeared to be activated, while those involving arachidonic acid were down-regulated. The authors concluded that these methods could be used as a rapid screening process for the detection of aristolochic acid ingestion. Chen and a different group of collaborators have also reported a metabolomic study of *Trypterygium wilfordii* (Celastraceae) using a similar approach [147]. This

TCM preparation is used to treat rheumatoid arthritis and other inflammatory conditions, but is known to have a number of undesirable side effects such as infertility and renal failure. Investigation of rat urine using GC- and LC-MS along with histological studies of the kidney, liver and testis revealed a time-dependent toxic effect at higher doses. Perturbations in metabolites related to energy status, amino acid processing and choline processing were observed. Other urinary metabolites observed suggested that the gut microflora populations were also affected by the extract.

There are several reports in the literature of metabolomic studies involving plants not considered to be toxic, but which are still of medicinal interest. Nicholson's lab has studied the widely consumed chamomile tea (*Matricaria recutita*, Asteraceae) in humans [148]. Using ^{1}H NMR spectroscopy to study metabolites in urine, these researchers identified non-trivial variations by gender and individual. In spite of these variations, however, they were able to identify increases in hippurate and glycine and decreases in creatinine as markers of chamomile tea ingestion; these changes in urinary metabolites were likely the result of changes in the gut microflora. Interestingly, these alterations persisted for 2 weeks after tea consumption was halted. Nicholson's lab has also reported two studies using ^{1}H NMR spectroscopic investigation of human plasma in subjects who had consumed soy isoflavones [149, 150]. These researchers identified clear differences in lipoprotein, amino acid and carbohydrate profiles resulting from the isoflavone consumption. As a final example, Ong's group mentioned earlier in connection with a proteomic study has also reported a metabolomic study on green tea consumption [151]. This comprehensive study combined GC-MS, LC-MS and ^{1}H NMR spectroscopic studies on human urine, and demonstrated significant changes in metabolites originating in energy and amino acid pathways immediately after ingestion of green tea.

Although really just beginning, systems toxicological studies such as the ones summarized here have tremendous potential. The reader has no doubt noticed that many different genes, proteins and metabolites are affected in a typical study. This is both the advantage and the weakness: holistic approaches are powerful but great effort must be expended to interpret the resulting data sets. It is likely that many researchers will gravitate to metabolomic studies, as these reflect the end result of changes in the transcriptome and proteome, and hence no speculation about what changes in a given mRNA level might ultimately mean is necessary. Metabolomics is also conceptually closest to traditional clinical chemical measurements, such as lipid panels, and as such may be more palatable to practicing clinicians.

Concluding remarks

The study of medicinal and toxic plants from any angle is fascinating, and motivates several disciplines. Although chemistry and toxicology began to-

gether, chemists have developed a uniquely molecular, historical and practical perspective on toxic plants. The Chemist's Perspective contributes both a supporting role, namely the practical synthesis of useful molecules, and an integrative role, one which connects phylogenetics to the biosynthesis of toxic molecules, and one which enables and bridges the different facets of systems toxicology.

Acknowledgments
This chapter is dedicated to the premier Costa Rican scientist Luis Diego Gómez, a good friend and my mentor in all things ethnobotanical. This project would not have been possible without the patience, support and encouragement of my wife Leslie. Special thanks go to Ms. Robin DiRocco for her excellent administrative support, and to the library staff at DePauw University, especially Ms. Mandy Henk. Their perseverance in locating yet another article for this project was invaluable. My son Keith A. Hanson made valuable suggestions on a draft.

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