NPR

Cite this: *Nat. Prod. Rep.*, 2011, **28**, 1261

www.rsc.org/npr

REVIEW

The saponins – polar isoprenoids with important and diverse biological activities

Anne Osbourn,*[a] Rebecca J. M. Goss[b] and Robert A. Field[c]

Received 21st February 2011
DOI: 10.1039/c1np00015b

Covering: up to the end of 2010

Saponins are polar molecules that consist of a triterpene or steroid aglycone with one or more sugar chains. They are one of the most numerous and diverse groups of plant natural products. These molecules have important ecological and agronomic functions, contributing to pest and pathogen resistance and to food quality in crop plants. They also have a wide range of commercial applications in the food, cosmetics and pharmaceutical sectors. Although primarily found in plants, saponins are produced by certain other organisms, including starfish and sea cucumbers. The underexplored biodiversity of this class of natural products is likely to prove to be a vital resource for discovery of high-value compounds. This review will focus on the biological activity of some of the best-studied examples of saponins, on the relationship between structure and function, and on prospects for synthesis of "designer" saponins.

1 Introduction

Saponins are a large and structurally diverse group of bioactive natural products that are found primarily in plants, most commonly within the dicots.[1–5] They also occur in certain marine organisms, such as starfish[6,7] and sea cucumbers.[8] These molecules are amphipathic glycosides with triterpene or steroid backbones. Their name is derived from *sapo*, the Latin word for soap, because they have surfactant properties and form stable, soap-like foams when shaken in aqueous solution. The names of some saponin-producing plant species – for example, soapwort (*Saponaria officinalis*), soapberry (*Sapindus* species) and soapbark (*Quillaja saponaria*) - reflect their original use as sources of natural soaps.

Saponins are isoprenoids (specifically triterpene- or steroid-derived) and are synthesised from mevalonate *via* farnesyl diphosphate and squalene (Scheme 1). This biosynthetic pathway is primarily cytosolic, in contrast to the plastid-localised methyl-erythritol-phosphate (MEP) pathway, which is the source of monoterpenes, diterpenes, tetraterpenes (carotenoids) and poly-prenols.[9] There are therefore fundamental differences in the biosynthetic routes to these various classes of isoprenoids (which are also collectively referred to as terpenes).

The tremendous range of structural diversity and biological activities represented within this major class of natural product renders saponins of particular interest for the development of new compounds for industrial, agricultural and pharmaceutical applications. The structural complexity of these molecules, coupled with the problems of detection, isolation and purification from nature and the challenges of chemical synthesis, has made it difficult to carry out definitive tests of structure–activity relationships that will drive the development of lead compounds for commercial applications. However advances in synthetic and semi-synthetic chemistry and in our understanding of saponin biosynthetic processes are now opening up new opportunities for exploitation of this important class of molecules, with the potential to create new-to-nature saponins with novel bioactivities.

[a]*Department of Metabolic Biology, John Innes Centre, Norwich Research Park, Norwich, NR4 7UH, UK. E-mail: anne.osbourn@bbsrc.ac.uk; Fax: +44 (1603) 450014; Tel: +44 (1603) 450407*
[b]*School of Chemistry, University of East Anglia, Norwich Research Park, Norwich, NR4 7TJ, UK*
[c]*Department of Biological Chemistry, John Innes Centre, Norwich Research Park, Norwich, NR4 7UH, UK*

2 Biological activities

Collectively saponins have a wide range of biological activities. This is, perhaps, not surprising given their immense structural diversity. Many of these molecules have antimicrobial, anti-herbivore and/or cytotoxic activity and their role in nature is likely to be in defense against pathogens, pests and predators. In plants, saponins appear to act as pre-formed antimicrobial barriers to pathogen attack but can also function as suppressors of induced defence responses following hydrolysis.[10–13] Genetic analysis of the wild crucifer *Barbarea vulgaris* suggests that resistance to flea beetle is positively correlated with triterpene glycoside content.[14] In sea cucumbers direct tissue mass spectrometry profiling and Matrix-Assisted Laser Desorption/Ionization–Mass Spectrometry (MALDI) Imaging have revealed stress-related accumulation of triterpene glycosides in the Cuvierian tubules, mechanical defense structures located in the posterior of the animal that are ejected towards a predator in response to aggression.[8] Saponins may also affect palatability of crop plants for animals, including humans. They have been linked with undesirable flavours in pea, and bitterness and anti-feedant effects in alfalfa and soybean.[15–17] High levels of steroidal glycoalkaloids are associated with toxicity to humans and need to be carefully monitored in food crops such as potatoes and tomatoes.[18–20]

The ability of saponins to complex with sterols and cause membrane permeabilisation is well known. However it is becoming increasingly clear that these molecules can also have a variety of other effects on cells that are mediated through specific interactions with metabolic processes, cellular receptors and structural proteins.[21–25] It is not possible to cover this vast array of activities here but only to highlight a few recent developments. For example, the triterpene glycoside avicin D (Fig. 1) and the dammarane saponin ginsenoside Rh2 (Fig. 1) have recently been shown to activate apoptosis by triggering Fas-mediated cell death through interference with membrane lipid rafts.[26,27] The pentacyclic triterpene lupeol (Fig. 1) also impacts on apoptosis, and has multiple effects on different components of the β-catenin signalling pathway.[28–30] β-Catenin is required for cell proliferation, cell–cell adhesion and development in animals. Interestingly, lupeol has also been implicated in suppression of cell proliferation in plants during the formation of nitrogen-fixing nodules in interactions with symbiotic bacteria, suggestive of commonalities in the effects of this simple triterpene in animal and plant cells.[31]

Triterpenes, such as oleanolic, ursolic and betulinic acid (Fig. 1), are highly selective and potent agonists of the G-protein coupled bile receptor TGR5, and have an anti-hyperglycaemic effect.[32] TGR5 is emerging as an attractive target for the treatment of metabolic disorders.[33] Other G-protein-mediated effects include stimulation of the sugar taste receptor in the blowfly, *Phormia regina*, by the legume triterpene saponin, chromosaponin I (sometimes also referred to as soyasaponin VI) (Fig. 1).[34]

Anne Osbourn

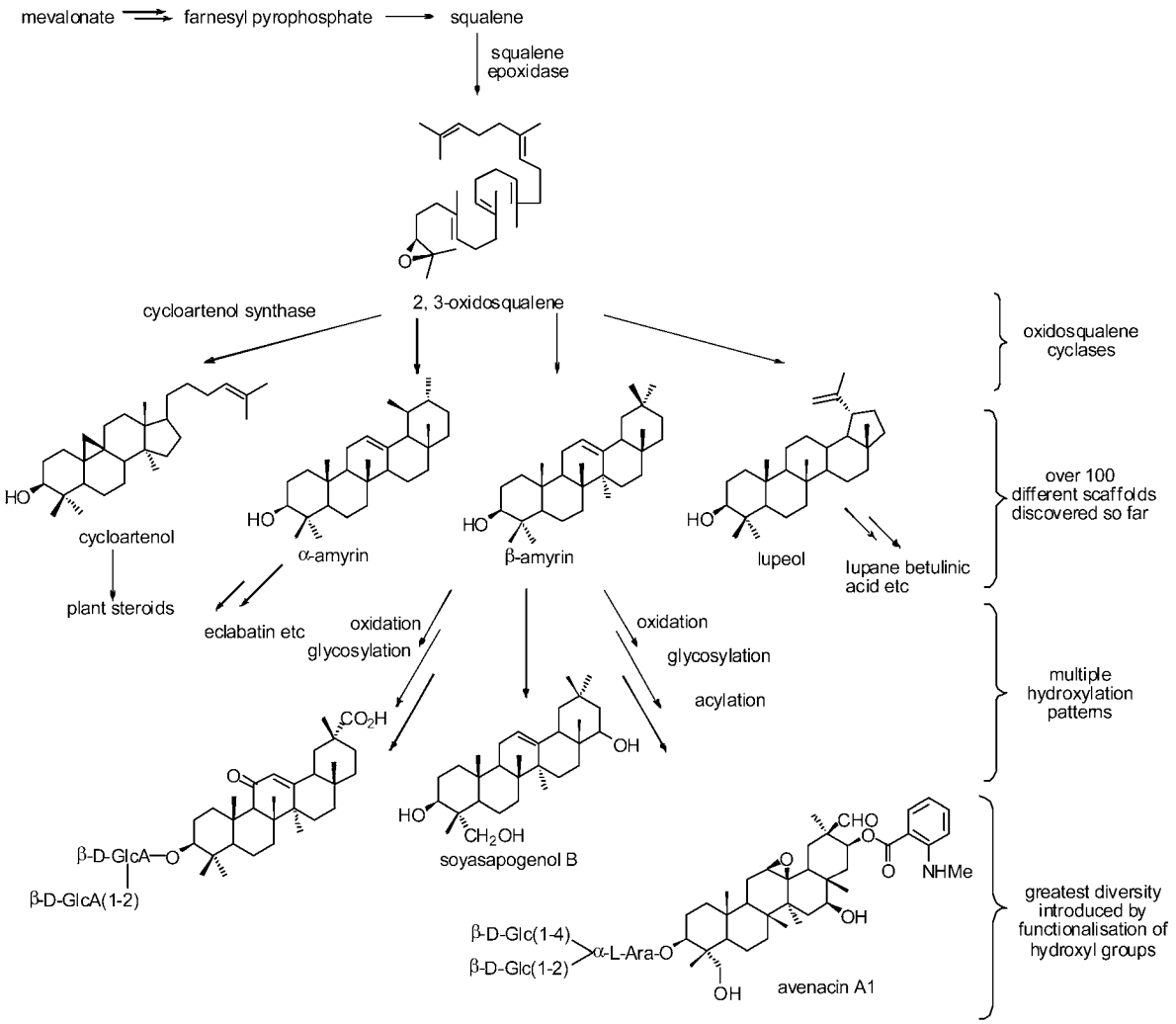
Anne Osbourn received her B. Sc. in Botany from the University of Durham in 1982 and her Ph.D. in Genetics from the University of Birmingham in 1985. She then moved to Norwich, where she is currently an Associate Research Director of the John Innes Centre and an honorary professor in the School of Biological Sciences, University of East Anglia (UEA). In 2005, while on sabbatical in the School of Literature and Creative Writing at UEA, Anne founded the Science, Art and Writing (SAW) Initiative, a creative science education initiative that encourages people of all ages to discover and explore science (www.sawtrust.org).

Rebecca Goss

Rebecca Goss (born 1976) is a senior lecturer in Organic Chemistry at the University of East Anglia. Rebecca carried out Ph.D. studies with Professor David O'Hagan at the University of Durham, before spending just under two years as a post-doctoral research associate in the laboratories of Professors Peter Leadlay and Jim Staunton at the University of Cambridge. In 2003 she took a lectureship at the University of Nottingham, before moving in close succession to the Universities of Exeter and then East Anglia, funded by a Royal Society BP Dorothy Hodgkin Fellowship. The Goss group have interests in medicinally relevant natural products and in their biosynthetic elucidation and manipulation. Rebecca also enjoys running, hill walking and painting.

Robert A. Field

Rob Field is a University of East Anglia graduate (1986) and PhD (1989; Dr A. H. Haines). Following postdoctoral work in Oxford (1989–91; Prof. J. E. Baldwin), Dundee (1992–94; Prof. M. A. J. Ferguson and Prof. S. W. Homans) and Alberta (1994; Prof. O. Hindsgaul), he was appointed to the faculty at the University of St Andrews in 1994, where he was promoted through the ranks to Professor (1999). He returned to Norwich in 2001, initially at UEA and latterly at the John Innes Centre. His research programme focuses on plant and microbial carbohydrate chemistry, in particular cell wall biosynthesis, starch metabolism and natural product biosynthesis.



Scheme 1 Overview of the routes to saponin biosynthesis and structural diversification (Ara – arabinose; Glc – glucose; GlcA – glucuronic acid).

It remains to be seen whether the bitter tastes of other legume saponins, such as 2,3-dihydro-2,5-dihydroxy-6-methyl-4H-pyran-4-one (DDMP)-containing saponins,[17] are mediated in similar ways.

Interestingly, triterpene saponins have been proposed to act as allelochemicals because some of these molecules have phytotoxic properties. In contrast, chromosaponin I has growth promoting effects on other plants[35–37] that are apparently mediated through regulation of auxin influx.[38] These diametrically opposed activities of structurally related molecules on plant growth open up further questions about whether growth inhibition/promotion occurs *via* different pathways or through antagonistic effects on a common pathway.

Given their structural similarity to membrane sterols, it is perhaps not surprising that triterpene and steroidal molecules can influence membrane-related processes within cells. For example, accumulation of incompletely glycosylated triterpene saponins in oat mutants leads to disruption of membrane trafficking with associated effects on plant growth and development,[39] while the steroidal alkaloid aglycone tomatidine (Fig. 1) inhibits sterol biosynthesis in yeast.[21] Given that glycosylation is a strategy that is commonly used by plants to inactivate natural products for storage purposes, it is interesting to consider the nature of the bioactive properties that a single triterpene or steroid may exhibit, from the aglycone skeleton through different degrees of glycosylation to the "finished" molecule, and the ramifications of these different levels of bioactivity for pathway evolution.

3 Towards commercial applications

The commercial applications of saponins are many and varied. These molecules are used as foaming agents in the beverage, food and cosmetics industries. Other uses relating to products for human consumption are as preservatives, flavour modifiers and cholesterol-lowering agents.[40] For example, the sweetness of liquorice roots is attributable to the presence of the triterpenoid saponin glycyrrhizin.[41] The emerging evidence for the health benefits of saponins is attracting increasing commercial attention, with expanding applications in the food, cosmetics, and pharmaceutical sectors. However, major challenges remain to be addressed with regard to the production of sufficiently well characterized materials at scale.[40]

With limited availability of pure materials from biological sources in many cases, chemical synthesis of saponins[42] has a key role to play in confirmation of the bioactive component of

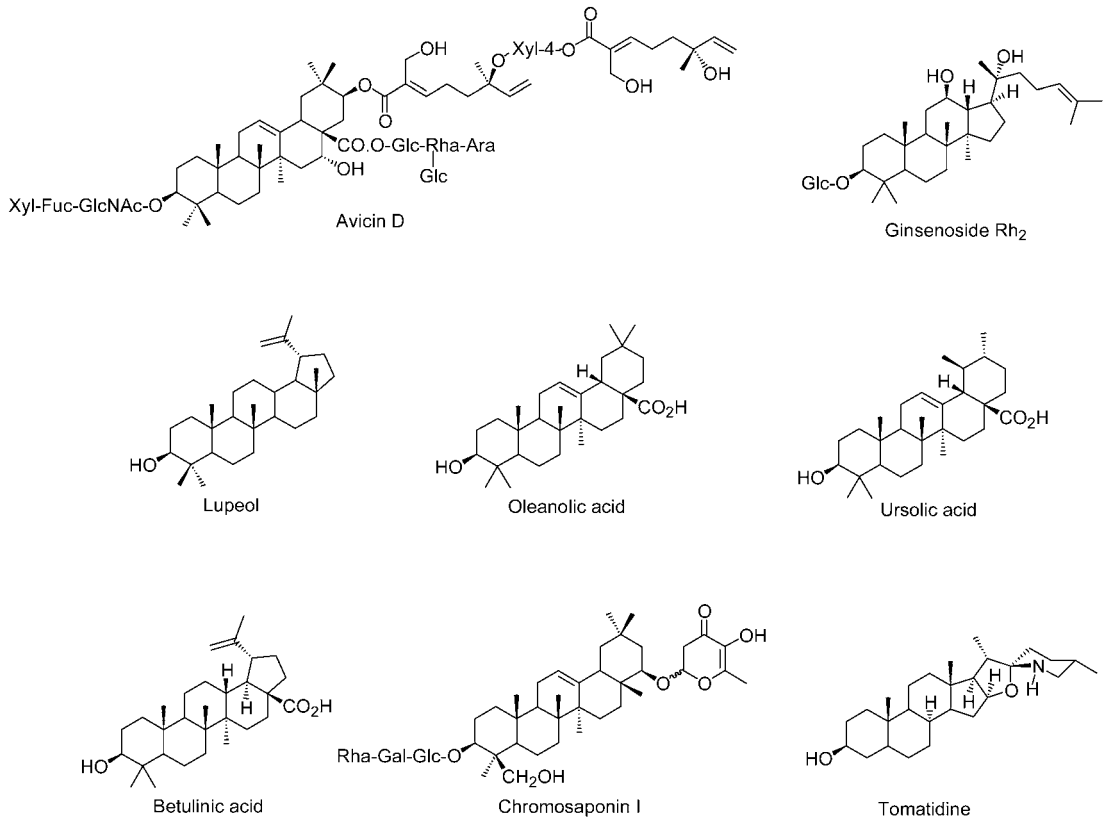


Fig. 1 Assorted bioactive saponins (Fuc – fucose; Gal – galactose; Glc – glucose; GlcA – glucuronic acid; GlcNAc – *N*-acetylglucosamine; Xyl – xylose).

complex saponin mixtures that occur in nature. For instance, elegant multi-step syntheses devised by the Gin lab[43–46] have provided valuable homogenous samples of the QS-21 saponins (apiose and xylose forms) (Fig. 2), normally isolated from the bark of *Quillaja saponaria* and widely used as an immunological adjuvant for vaccination. The unique capacity of QA-21 saponins to stimulate both the Th1 immune response and the production of cytotoxic T lymphocytes against exogenous antigens makes them attractive adjuvants, but serious drawbacks associated with scarcity, difficulty in purification to

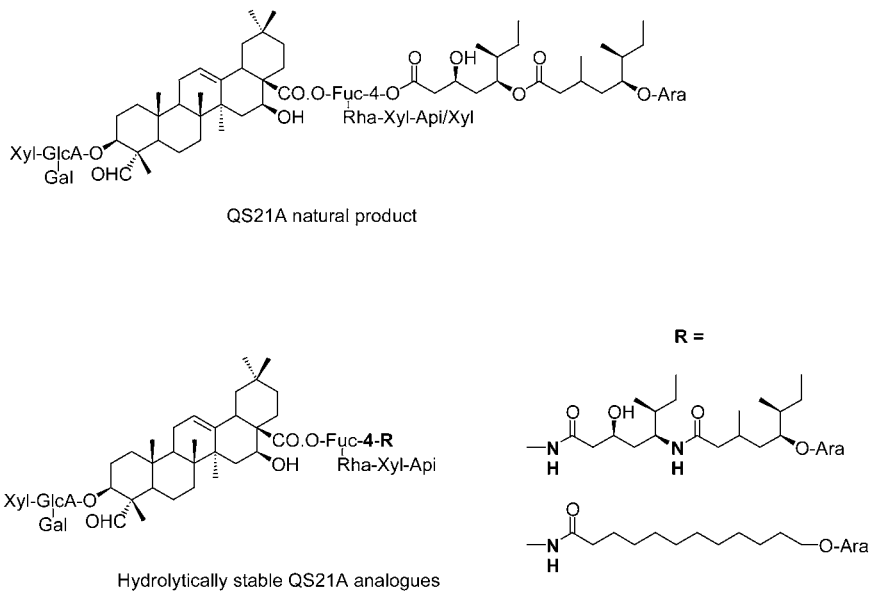
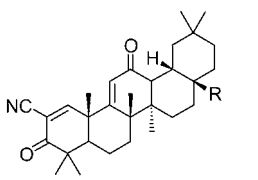


Fig. 2 Natural and semi-synthetic QS21A adjuvants (Api – apiose; Ara – arabinose; Fuc – fucose; Gal – galactose; GlcA – glucuronic acid; Rha – rhamnose; Xyl – xylose).

homogeneity, haemolytic side-effects, high toxicity and poor aqueous stability currently limit their clinical use.[47] As Gin notes, developments in chemical synthesis "lay the foundation for future exploration of structure–function correlations to enable the discovery of novel saponins with increased potency, enhanced stability, and attenuated toxicity".[48] With robust synthetic methods in hand, the scope to develop amide-modified QS-21 analogues (Fig. 2) with improved properties has been nicely demonstrated.[49] Several other medicinal plants also produce saponins that have adjuvant activity but that are structurally distinct from QS-21.[47] For example, ginsomes are nanoparticles containing ginsenosides, triterpene saponins from ginseng (*Panax ginseng*) that promote IgG responses in mice.[50]

The wide-ranging pharmacological properties of liquorice-derived glycyrrhizic acid (GA: anti-inflammatory, antiviral, antimicrobial, antioxidative, anticancer, immunomodulatory, hepatoprotective and cardioprotective) are attracting much interest. For instance, the chemical modification of GA shows promise for the development of novel antiviral agents for the prophylaxis and treatment of HIV, hepatitis B and C, coronavirus and herpes simplex virus infections,[51] while a number of other saponins also show antimicrobial properties.[52] A number of triterpene scaffolds possess anti-HIV and antitumor activities, including the lupanes, ursanes, oleananes, lanostanes and dammaranes,[53,54] while the dietary pentacyclic triterpene lupeol (Fig. 1) displays anti-inflammatory and anti-cancer properties.[30] In terms of exploiting the diverse saponin structures that display anti-cancer properties, presumably by targeting a range of different metabolic pathways,[55] the synthetic oleanane triterpenoids (Fig. 3) represent a promising new class of multifunctional drug. These compounds possess unique molecular and cellular mechanisms of action that are not dependent on either classical cytotoxic activity or the unique targeting of single metabolic steps.[56] These semi-synthetic compounds are potent anti-proliferative and pro-apoptotic agents that impact on inflammation and the redox state of cells and tissues and have been shown to prevent, or can be used to treat, cancer in experimental animals. For instance, 1-[2-cyano-3-, 12-dioxooleana-1,9(11)-dien-28-oyl] imidazole (CDDO-Im) (Fig. 3) has been assessed in mouse models of chronic obstructive pulmonary disease, which includes emphysema and chronic bronchitis resulting from prolonged exposure to cigarette smoke. This compound, which significantly reduces oxidative stress of the lung and pulmonary hypertension, has potential for the treatment of this major public health burden for which there is currently no effective treatment.[57] An oleanolic acid derivative, bardoxolone methyl (CDDO-methyl ester) (Fig. 3) has also been shown to activate the transcription factor Nrf2 with associated increased production of antioxidant enzymes, resulting in inhibition of pro-inflammatory transcription factors involved in carcinogenesis.[58]



CDDO-Im, R = CO.imidazole
CDDO-methyl ester, R = CO_2Me

Fig. 3 Bioactive synthetic oleananes (see Fig. 1 for related naturally occurring structures).

4 Discovery of new enzymes, pathways and compounds

Despite the many and varied applications of saponins, the biosynthesis of these compounds has remained poorly characterised until very recently. Saponin generation is initiated by the oxidation of squalene to 2,3-oxidosqualene (Scheme 1) by squalene epoxidase. Cyclisation of 2,3-oxidosqualene by specific oxidosqualene cyclases results in the generation of over 100 scaffolds, including β-amyrin, α-amyrin, and lupeol (Scheme 1); the terpenoid core is subsequently modified by a series of oxidoreductase enzymes. The hydroxylated species are then elaborated by an array of glycosyl and acyl transferases. The plethora of glycosylation patterns is the greatest contributor to structural and biological diversity amongst this large and diverse class of natural products.

4.1 Oxidosqualene cyclases

Oxidosqualene cyclases (OSCs) catalyse and control a series of exquisite carbocation cascades mediating the formation of the triterpenoid tetra or pentacyclic cores. Whilst the cycloartenol synthases, responsible for primary steroid metabolism in plants, are highly conserved across a wide variety of species; the OSC family has expanded greatly in plants and a divergent series of plant OSCs have been shown to mediate the formation of a diverse series of non-steroidal triterpenoid cores. The chemistry and enzymology of squalene cyclases and OSCs have been extensively reviewed elsewhere.[3,59] One notable advance in this area is the careful study by Lodeiro *et al.*[60] demonstrating that one OSC baruol synthase (BARS1) from *Arabidopsis thaliana* is, in its unmodified form, capable not only of mediating the generation of baruol, but also of 22 other minor products of alternative cyclisation pathways involving deprotonation and carbocation formation at 14 different sites across the 5 rings. This study dispels the previous misconception of tight control in OSCs.[60]

4.2 Oxidoreductases

The subsequent steps in saponin biosynthesis have been less well characterised. Glycyrrhizin is a commercially important compound used world-wide in healthcare and as a low calorie sweetener, resulting in global trade in liquorice root being estimated at over $42.1M per year.[61] Glycyrrhizin consists of the calcium and potassium salts of glycyrrhizic acid (Scheme 1), a triterpenoid saponin. The commercial significance of this compound has resulted in a more focused analysis of its biosynthesis, which is believed to start with the generation of β-amyrin (Scheme 1), followed by oxidation at C-11 and C-30, then subsequent glycosylation of the hydroxyl of C-3. Transcript profiling of a set of liquorice expressed sequence tags (ESTs) has recently enabled the identification of CYP 88D6, a cytochrome P450, capable of mediating the sequential two step oxidation of

β-amyrin at C-11. Co-expression of CYP88D6 with β-amyrin in yeast has resulted in the generation of an identical oxidation product.[62]

The avenacins, saponins found in the epidermal root cells of oat, have attracted attention due to their ability to confer broad spectrum resistance to fungal pathogens. These triterpenoid saponins have been demonstrated to be derived from β-amyrin, which is oxidised at C-16, C-21, C-23 and C-30 and an epoxide is introduced at C-12, C-13. As with glycyrrhizin, the hydroxyl of C-3 is glycosylated. A unique feature of the avenacins is the acylation of the hydroxyl that is introduced at C-21. The cytochrome P450 enzyme CYP51H10 (SAD2), has been demonstrated to be involved in the biosynthesis of the avenacins (Qi, 2006; Qin *et al.*, 2010),[63,64] however its exact role has not yet been pinpointed. The CYP51 family are believed to be the most ancient and highly conserved members of the P450 superfamily, and were previously known only to catalyse C-14 demethylation in primary steroid biosynthesis. SAD2 belongs to the newly defined CYP51H divergent subfamily, and is the first CYP51 to have been shown to have a function other than C-14 demethylation.[65] The CYP51H subfamily appears to be restricted to monocots.[63] A C-24 β-amyrin hydroxylase, CYP93E1, has been identified in soybean (*Glycine max*).[66] Two P450s involved in the biosynthesis of thalianol in Arabidopsis, CYP708A2 and CYP705A5 have been identified.[67] A number of P450s implicated in the biosynthesis of saponins by the model legume *Medicago truncatula* have been found using genomic and co-expression analysis, although these enzymes remain to be characterised and their exact roles determined.[68]

4.3 Glycosyltransferases

Glycosylation patterns in natural products are commonly key to bioactivity. Glycosyltransferases tend to have fairly broad substrate specificities, and it can be difficult to pinpoint the true natural substrate using enzyme kinetics and gene knock out approaches. Though glycosyltransferases play the greatest role in introducing structural and biological diversity to this class of natural products, they have so far remained relatively uncharacterised. Mutants of oat that are affected in triterpene glycosylation have been isolated but the corresponding genes have not yet been cloned.[39]

An elegant study combining DNA array-based and *in silico* transcript profiling with targeted metabolite profiling has enabled the identification of glycosyltransferases implicated in saponin synthesis in *M. truncatula*.[69] Further studies in this system utilising the wound signal methyl jasmonate, which has previously been demonstrated to elevate saponin production,[70] enabled analysis of induced transcript expression patterns, so allowing further glycosyltransferases involved in saponin biosynthesis to be identified.[68] *In vitro* mutagenesis has been utilised to identify the amino acid residues key to activity of glycosyltransferases involved in the biogenesis of saponins in solanaceous plants, and to engineer function into an inactive homologue.[71] The recent characterisation of two glycosyltransferases involved in the biosynthesis of soyasaponin I in *Glycine max* (soybean) implicates the successive addition of sugars rather than transfer of a preformed polysaccharide to an aglycone.[66]

4.4 Acyl transferases

Numerous plant natural products, including the saponins, are modified by the incorporation of an acyl group, and acylation not only contributes to structural diversity, but also impact on function. The BADH family of acyltransferases from plants have been well characterised. Studies have been carried out on over 40 BAHD acyltransferases, which utilise CoA thioesters as acyl donors.[72] A more recently discovered class of plant acyltransferases are the serine carboxypeptidase-like proteins (SCPLs), which utilise *O*-glucosyl esters as acyl donors.[73–77] An unusual feature of the avenacins (Scheme 1) is their fluorescence under UV light, which is due to the incorporation of an *N*-methyl anthranilate moiety. This curious property has enabled the development of high throughput assays to rapidly screen for oat mutants deficient in the biosynthesis of these compounds.[10,63,64] Oat SCPL1 (SAD7) has been shown to be able to transfer both *N*-methyl anthranilate and benzoate onto the hydroxyl at C-21 of modified β-amyrin in the biosynthesis of the avenacins.[78]

4.5 Gene clusters for triterpene synthesis

Genes for well-characterised natural product pathways in plants (*e.g.* anthocyanins, glucosinolates) are generally unlinked. However, it transpires that the genes for the synthesis of avenacins are organised in an "operon-like" gene cluster in oat (*i.e.* these genes are contiguous in the oat genome and are co-regulated).[63] A second gene cluster for triterpene synthesis (the thalianol cluster) has also recently been discovered in thale cress (*Arabidopsis thaliana*).[67] These clusters have evolved relatively recently in evolutionary time through independent events within these two plant lineages.[67,79] These findings suggest that triterpene pathways may be predisposed to gene clustering for reasons that are as yet unclear, although further information about the genomic organisation of other triterpene pathways is required before more general conclusions can be drawn.

5 Future prospects - towards designer saponins through synthetic chemistry and biology

Structure–activity relationships are beginning to emerge for some saponins. For instance, the main structural features responsible for enhancement of cytotoxicity of oleananes are a free carboxyl group at C-28, a free hydroxyl group at C-16 and acylation at C-21 or C-22. The presence of α-L-Ara*p* as the first sugar attached at C-3 in oleananes and α-L-Rha*p* in lupanes is also important.[25] Such information prompts the search for methods to access specific, homogeneous saponin natural products and new-to-nature variants thereof. In turn, this presents opportunities for synthetic chemistry, biochemistry (a triterpene/saponin toolkit of genes and enzymes) and semi-synthetic chemistry (pushing the limits of the enzyme toolkit to incorporate non-natural groups). Projecting ahead, one can envisage synthetic biology approaches, for instance based on pathway transfer between species, with either yeast (*Saccharomyces cerevisiae*) or plant systems as hosts for the assembly of multiple steps in saponin synthesis, as illustrated by the production of precursors to the sesquiterpene artmesinin in yeast, for instance.[80] Manipulation of saponin content through plant breeding or genetic modification is expected to lead to the

development of crop varieties with improved traits (for example, with enhanced resistance to pathogens and herbivores or with reduced anti-feedant properties, improved palatability or enhanced flavour). It also presents opportunities for use of plants as green factories for the production of saponins or saponin pathway intermediates that are otherwise limiting and that may be exploited for commercial use in various ways.

In the short term, and from a chemistry perspective, natural products continue to stimulate interest from the drug discovery community[81] and provide inspiration for the development of asymmetric catalysis methodologies[82] and for the selective oxidation of unactivated C–H bonds – a prevalent feature of triterpenes – by either chemical (*e.g.* ref. 83 and 84) or biologically-inspired oxidation catalysts.[85] Cytochrome P450s also offer scope for the selective oxidation of hydrocarbon structures, leading to alcohols, or through the sequential action of a P450 and a fluorinating agent to give the corresponding fluorinated compounds,[86] which are highly prized by the pharmaceutical industry.[87] The biological introduction of halogens is also now achievable through heterologous expression of microbial halogenases, for instance providing access to a fluorinated version of the chlorinated natural product salinosporamide A.[88] Transfer of the chlorination biosynthetic machinery from a soil bacterium to the medicinal plant *Catharanthus roseus* (Madagascar periwinkle) provides access to chlorinated alkaloids,[89] while transfer of the halogenase gene from pyrrolnitrin biosynthesis into *Streptomyces coeruleorubidus* resulted in efficient *in situ* chlorination of the uridyl peptide antibiotic pacidamycin. In the latter case, the installed chlorine atoms provide chemically-addressable handles for further elaboration through palladium-mediated cross-coupling chemistry.[90]

Only time will tell if these contemporary approaches will find utility in the saponin area, although the increasing range of exploitable properties attributable to this class of natural products will undoubtedly encourage further exploration of triterpene chemistry and biology.

6 Acknowledgements

We thank Melissa Dokarry for provision of the image used in the graphical abstract.

7 References

1 A. Marston and K. Hostettmann, *Phytochemistry*, 1985, **24**, 639–652.
2 J. D. Connolly and R. A. Hill, *Nat. Prod. Rep.*, 2005, **22**, 487–503.
3 D. R. Phillips, J. M. Rasbery, B. Bartel and S. P. Matsuda, *Curr. Opin. Plant Biol.*, 2006, **9**, 305–314.
4 J. P. Vincken, L. Heng, A. de Groot and H. Gruppen, *Phytochemistry*, 2007, **68**, 275–297.
5 A. C. A. Yendo, F. de Costa, G. Gosmann and A. G. Fett-Neto, *Mol. Biotechnol.*, 2010, **46**, 94–104.
6 H.-W. Liu, J. K. Li, D. W. Zhang, J. C. Zhang, N. L. Wang, G. P. Cai and X. S. Yao, *J. Asian Nat. Prod. Res.*, 2008, **10**, 521–529.
7 H. F. Tang, G. Cheng, J. Wu, X. L. Chen, S. Y. Zhang, A. D. Wen and H. W. Lin, *J. Nat. Prod.*, 2009, **72**, 284–289.
8 S. Van Dyck, P. Gerbaux and P. Flammang, *Mar. Drugs*, 2010, **8**, 173–189.
9 J. Chappell, *Curr. Opin. Plant Biol.*, 2002, **5**, 151–157.
10 K. Papadopoulou, R. E. Melton, M. Leggett, M. J. Daniels and A. E. Osbourn, *Proc. Natl. Acad. Sci. U. S. A.*, 1999, **96**, 12923–12928.
11 K. Bouarab, R. Melton, J. Peart, D. Baulcombe and A. Osbourn, *Nature*, 2002, **418**, 889–892.
12 S.-I. Ito, T. Eto, S. Tanaka, N. Yamauchi, H. Takahara and T. Ikeda, *FEBS Lett.*, 2004, **571**, 31–34.
13 P. Bednarek and A. Osbourn, *Science*, 2009, **324**, 746–748.
14 V. Kuzina, C. T. Ekstrom, S. B. Andersen, J. K. Nielsen, C. E. Olsen and S. Bak, *Plant Physiol.*, 2009, **151**, 1977–1990.
15 J. Milgate and D. C. K. Roberts, *Nutr. Res.*, 1995, **15**, 1223–1249.
16 R. A. Dixon and L. W. Sumner, *Plant Physiol.*, 2003, **131**, 878–885.
17 L. Heng, J. P. Vincken, G. van Koningsveld, A. Legger, H. Gruppen, T. van Boekel, J. Roozen and F. Voragen, *J. Sci. Food Agric.*, 2006, **86**, 1225–1231.
18 M. Friedman, *J. Agric. Food Chem.*, 2002, **50**, 5751–5780.
19 Y. I. Korpan, E. A. Nazarenko, I. V. Skryshevskaya, C. Martelet, N. Jaffrezic-Renault and A. V. El'skaya, *Trends Biotechnol.*, 2004, **22**, 147–151.
20 M. Friedman, *J. Agric. Food Chem.*, 2006, **54**, 8655–8681.
21 V. Simons, J. P. Morrissey, M. Latijnhouwers, M. Csukai, A. Cleaver, C. Yarrow and A. Osbourn, *Antimicrob. Agents Chemother.*, 2006, **50**, 2732–2740.
22 F. M. Siu, D. L. Ma, Y. W. Cheung, C. N. Lok, K. Yan, Z. Q. Yang, M. S. Yang, S. X. Xu, B. C. B. Ko, Q. Y. He and C. M. Che, *Proteomics*, 2008, **8**, 3105–3117.
23 A. Weng, C. Bachran, H. Fuchs and M. F. Melzig, *Chem.-Biol. Interact.*, 2008, **176**, 204–211.
24 S. R. Wang and W. S. Fang, *Curr. Top. Med. Chem.*, 2009, **9**, 1581–1596.
25 I. Podolak, A. Galanty and D. Sobolewska, *Phytochem. Rev.*, 2010, **9**, 425–474.
26 Z. X. Xu, T. Ding, V. Haridas, F. Connolly and J. U. Gutterman, *PLoS One*, 2009, **4**, e8532.
27 J. S. Yi, H. J. Choo, B. R. Cho, H. M. Kim, Y. N. Kim, Y. M. Ham and Y. G. Ko, *Biochem. Biophys. Res. Commun.*, 2009, **385**, 154–159.
28 J. Behrens, *Ann. N. Y. Acad. Sci.*, 2000, **910**, 21–35.
29 M. Conacci-Sorrell, J. Zhurinsky and A. Ben-Ze'ev, *J. Clin. Invest.*, 2002, **109**, 987–991.
30 M. Saleem, *Cancer Lett.*, 2009, **285**, 109–115.
31 C. Delis, A. Krokida, S. Georgiou, L. M. Pena-Rodriguez, N. Kavroulakis, E. Ioannou, V. Roussis, A. E. Osbourn and K. Papadopoulou, *New Phytol.*, 2011, **189**, 335–346.
32 H. Sato, C. Genet, A. Strehle, C. Thomas, A. Lobstein, A. Wagner, C. Mioskowski, J. Auwerx and R. Saladin, *Biochem. Biophys. Res. Commun.*, 2007, **362**, 793–798.
33 A. Tiwari and P. Maiti, *Drug Discovery Today*, 2009, **14**, 523–530.
34 A. Ahamed, S. Tsurumi and T. Amakawa, *J. Insect Physiol.*, 2000, **48**, 367–374.
35 S. Tsurumi and S. Wada, *Plant Cell Physiol.*, 1995, **36**, 925–929.
36 S. Tsurumi and K. Ishizawa, *Plant Cell Physiol.*, 1997, **38**, 668–675.
37 S. Tsurumi, K. Ishizawa, A. Rahman, K. Soga, T. Hoson, N. Goto and S. Kamisaka, *J. Plant Physiol.*, 2000, **156**, 60–67.
38 A. Rahman, A. Ahamed, T. Amakawa, N. Goto and S. Tsurumi, *Plant Physiol.*, 2001, **125**, 990–1000.
39 P. Mylona, A. Owatworakit, K. Papadopoulou, H. Jenner, B. Qin, K. Findlay, L. Hill, X. Qi, S. Bakht, R. Melton and A. Osbourn, *Plant Cell*, 2008, **20**, 201–212.
40 Ö. Güçlü-Üstündag and G. Mazza, *LWT–Food Sci. Technol.*, 2008, **41**, 1600–1606.
41 I. Kitagawa, *Pure Appl. Chem.*, 2002, **74**, 1189–1198.
42 B. Yu and J. Sun, *Chem.–Asian J.*, 2009, **4**, 642–654.
43 K. Deng, M. M. Adams, P. Damani, P. O. Livingston, G. Ragupathi and D. Y. Gin, *Angew. Chem., Int. Ed.*, 2008, **47**, 6395–6398.
44 K. Deng, M. M. Adams and D. Y. Gin, *J. Am. Chem. Soc.*, 2008, **130**, 5860–5861.
45 Y. J. Kim, P. Wang, M. Navarro-Villalobos, B. D. Rohde, J. Derryberry and D. Y. Gin, *J. Am. Chem. Soc.*, 2006, **128**, 11906–11915.
46 P. Wang, Y. J. Kim, M. Navarro-Villalobos, B. D. Rohde and D. Y. Gin, *J. Am. Chem. Soc.*, 2005, **127**, 3256–3257.
47 H. X. Sun, Y. Xie and Y. P. Ye, *Vaccine*, 2009, **27**, 1787–1796.
48 G. Ragupathi, P. Damani, K. Deng, M. M. Adams, J. Hang, C. George, P. O. Livingston and D. Y. Gin, *Vaccine*, 2010, **28**, 4260–4267.
49 M. M. Adams, P. Damani, N. R. Perl, A. Won, F. Hong, P. O. Livingston, G. Ragupathi and D. Y. Gin, *J. Am. Chem. Soc.*, 2010, **132**, 1939–1945.
50 X. M. Song, L. M. Zhang and S. H. Hu, *Vaccine*, 2009, **27**, 2306–2311.
51 L. Baltina, R. Kondratenko, O. Plyasunova, A. Pokrovskii and G. Tolstikov, *Pharm. Chem. J.*, 2009, **43**, 539–548.

52 M. Saleem, M. Nazir, M. S. Ali, H. Hussain, Y. S. Lee, N. Riaz and A. Jabbar, *Nat. Prod. Rep.*, 2010, **27**, 238–254.
53 N. Sultana and A. Ata, *J. Enzyme Inhib. Med. Chem.*, 2008, **23**, 739–756.
54 R.-Y. Kuo, K. Qian, S. L. Morris-Natschke and K.-H. Lee, *Nat. Prod. Rep.*, 2009, **26**, 1321–1344.
55 S. Man, W. Gao, Y. Zhang, L. Huang and C. Liu, *Fitoterapia*, 2010, **81**, 703–714.
56 K. T. Liby, M. M. Yore and M. B. Sporn, *Nat. Rev. Cancer*, 2007, **7**, 357–369.
57 T. E. Sussan, T. Rangasamy, D. J. Blake, D. Malhotra, H. El-Haddad, D. Bedja, M. S. Yates, P. Kombairaju, M. Yamamoto, K. T. Liby, M. B. Sporn, K. L. Gabrielson, H. C. Champion, R. M. Tuder, T. W. Kensler and S. Biswal, *Proc. Natl. Acad. Sci. U. S. A.*, 2009, **106**, 250–255.
58 M. S. Yates, M. Tauchi, F. Katsuoka, K. C. Flanders, K. T. Liby, T. Honda, G. W. Gribble, D. A. Johnson, J. A. Johnson, N. C. Burton, T. R. Guilarte, M. Yamamoto, M. B. Sporn and T. W. Kensler, *Mol. Cancer Ther.*, 2007, **6**, 154–162.
59 I. Abe, *Nat. Prod. Rep.*, 2007, **24**, 1311–1331.
60 S. Lodeiro, Q. Xiong, W. K. Wilson, M. D. Kolesnikova, C. S. Onak and S. P. Matsuda, *J. Am. Chem. Soc.*, 2007, **129**, 11213–11222.
61 P.-M. Parker, *The world market for licorice roots: A 2007 global trade perspective*, ICON Group International Inc, San Diego.
62 H. Seki, K. Ohyama, S. Sawai, M. Mizutani, T. Ohnishi, H. Sudo, T. Akashi, T. Aoki, K. Saito and T. Muranaka, *Proc. Natl. Acad. Sci. U. S. A.*, 2008, **105**, 14204–14209.
63 X. Qi, S. Bakht, B. Qin, M. Leggett, A. Hemmings, F. Mellon, J. Eagles, D. Werck-Reichhart, H. Schaller, A. Lesot, R. Melton and A. Osbourn, *Proc. Natl. Acad. Sci. U. S. A.*, 2006, **103**, 18848–18853.
64 B. Qin, J. Eagles, F. A. Mellon, P. Mylona, L. Pena-Rodriguez and A. E. Osbourn, *Phytochemistry*, 2010, **71**, 1245–1252.
65 T. Ohnishi, T. Yokota and M. Mizutani, *Phytochemistry*, 2009, **70**, 1918–1929.
66 M. Shibuya, M. Hoshino, Y. Katsube, H. Hayashi, T. Kushiro and Y. Ebizuka, *FEBS J.*, 2006, **273**, 948–959.
67 B. Field and A. E. Osbourn, *Science*, 2008, **320**, 543–547.
68 M. A. Naoumkina, L. V. Modolo, D. V. Huhman, E. Urbanczyk-Wochniak, Y. Tang, L. W. Sumner and R. A. Dixon, *Plant Cell*, 2010, **22**, 850–866.
69 L. Achnine, D. V. Huhman, M. A. Farag, L. W. Sumner, J. W. Blount and R. A. Dixon, *Plant J.*, 2005, **41**, 875–887.
70 H. Suzuki, M. Reddy, M. Naoumkina, N. Aziz, G. May, D. Huhman, L. Sumner, J. Blount, P. Mendes and R. Dixon, *Planta*, 2005, **220**, 696–707.
71 A. Kohara, C. Nakajima, S. Yoshida and T. Muranaka, *Phytochemistry*, 2007, **68**, 478–486.
72 J. C. D'Auria, *Curr. Opin. Plant Biol.*, 2006, **9**, 331–340.
73 C. Lehfeldt, A. M. Shirley, K. Meyer, M. O. Ruegger, J. C. Cusumano, P. V. Viitanen, D. Strack and C. Chapple, *Plant Cell*, 2000, **12**, 1295–1306.
74 A. X. Li and J. C. Steffens, *Proc. Natl. Acad. Sci. U. S. A.*, 2000, **97**, 6902–6907.
75 A. M. Shirley, C. M. McMichael and C. Chapple, *Plant J.*, 2001, **28**, 83–94.
76 C. M. Fraser, M. G. Thompson, A. M. Shirley, J. Ralph, J. A. Schoenherr, T. Sinlapadech, M. C. Hall and C. Chapple, *Plant Physiol.*, 2007, **144**, 1986–1999.
77 D. Weier, J. Mittasch, D. Strack and C. Milkowski, *Planta*, 2008, **227**, 375–385.
78 S. T. Mugford, X. Qi, S. Bakht, L. Hill, E. Wegel, R. K. Hughes, K. Papadopoulou, R. Melton, M. Philo, F. Sainsbury, G. P. Lomonossoff, A. D. Roy, R. J. M. Goss and A. E. Osbourn, *Plant Cell*, 2009, **21**, 2473–2484.
79 A. Osbourn, *Trends Genet.*, 2010, **26**, 449–457.
80 D. K. Ro, E. M. Paradise, M. Ouellet, K. J. Fisher, K. L. Newman, J. M. Ndungu, K. A. Ho, R. A. Eachus, T. S. Ham, J. Kirby, M. C. Y. Chang, S. T. Withers, Y. Shiba, R. Sarpong and J. D. Keasling, *Nature*, 2006, **440**, 940–943.
81 K.-H. Lee, *J. Nat. Prod.*, 2010, **73**, 500–516.
82 J. T. Mohr, M. R. Krout and B. M. Stoltz, *Nature*, 2008, **455**, 323–332.
83 M. S. Chen and M. C. White, *Science*, 2007, **318**, 783–787.
84 K. Chen and P. S. Baran, *Nature*, 2009, **459**, 824–828.
85 L. Que and W. B. Tolman, *Nature*, 2008, **455**, 333–340.
86 A. Rentmeister, F. H. Arnold and R. Fasan, *Nat. Chem. Biol.*, 2009, **5**, 26–28.
87 K. Muller, C. Faeh and F. Diederich, *Science*, 2007, **317**, 1881–1886.
88 A. S. Eustaquio, D. O'Hagan and B. S. Moore, *J. Nat. Prod.*, 2010, **73**, 378–382.
89 W. Runguphan, X. Qu and S. E. O'Connor, *Nature*, 2010, **468**, 461–464.
90 A. D. Roy, S. Gruschow, N. Cairns and R. J. M. Goss, *J. Am. Chem. Soc.*, 2010, **132**, 12243–12245.

