REVIEW

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Anti-staphylococcal plant natural products

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Covering: 1995 to 2003

The occurrence of vancomycin-resistant *Staphylococcus aureus* (VRSA) and multidrug-resistant (MDR) strains of this organism necessitate the discovery of new classes of anti-staphylococcal drug leads. At present there are no single chemical entity plant derived antibacterials used clinically, and this biologically diverse group deserves consideration as a source of chemical diversity for two important reasons. Firstly, plants have exceptional ability to produce cytotoxic agents and, secondly, there is an ecological rationale that antibacterial natural products should be present or synthesised *de novo* following microbial attack to protect plants from invasive and pathogenic microbes in their environment. This review cites plant natural products that either modify resistance in *Staphylococcus aureus* or are antibacterial through bacteriostatic or bactericidal properties. The activities described here show that there are many potential new classes of anti-staphylococcal agents which should undergo further cytotoxicity, microbial specificity and preclinical *in vivo* studies to assess their potential.

Simon Gibbons was born in Gosport, England in 1966. He studied Chemistry at Kingston Polytechnic and completed a PhD in Phytochemistry under the supervision of Dr Sandy Gray and Professor Peter G. Waterman at the Department of Pharmaceutical Sciences, University of Strathclyde in 1994. He worked as a project chemist and research leader at the natural product biotech company Xenova before moving to the newly formed Faculty of Pharmacy at Kuwait University as Assistant Professor of Pharmaceutical Chemistry in 1997. In 1999 he moved to the University of London School of Pharmacy as Lecturer in Phytochemistry. His research is focused on the antibacterial and resistance modifying properties of plant derived natural products.

Simon Gibbons

1 Introduction

This review encompasses the literature from 1995 to 2003 on plant derived anti-staphylococcal compounds which act as either bacterial resistance modifying agents (RMAs) or have direct antibacterial action. For antibacterial compounds, only single chemical entities (SCEs) which have minimum inhibitory concentrations (MIC) of less than 64 μg ml^{-1} (or less than 5 μl ml^{-1} or 0.25% v/v for liquids) have been selected from the literature, and only examples where standard MIC determinations employing either broth or agar dilution methodologies are cited. These criteria have been applied so that direct comparison of activities can be made between compound classes. Extracts, whilst traditionally used in many systems of medicine to treat infections caused by bacteria, have been excluded from this review in an attempt to focus on the potential of plant derived SCEs.

The literature abounds with claims of natural products and extracts displaying antibiotic activity with many papers describing compounds with MIC values over 1 mg ml^{-1} (1000 μg ml^{-1}), which from a clinical perspective has little relevance. It is likely that a number of relatively inert substances may display antibacterial activity at this high concentration.

Resistance modifying agents are compounds which potentiate the activity of an antibiotic against a resistant strain. These compounds may for example, specifically target a resistance mechanism, such as the inhibition of multidrug resistance (MDR), *e.g.* inhibition of the NorA efflux mechanism in *Staphylococcus aureus*[1] or act in a synergistic fashion *via* an undescribed mechanism.

Staphylococcus aureus is a commensal organism that is commonly cited as being a major hospital-acquired pathogen.[2,3] Strains of this species that are resistant to β-lactams, notably the methicillin-resistant *Staphylococcus aureus* (MRSA) strains, have been described from clinical sources for over forty years.[4,5] It is the ability of this Gram-positive organism to acquire resistance to practically all useful antibiotics that is cause for considerable concern. Furthermore, in the UK there has been a significant increase in the number of death certificates which

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mention MRSA with 47 citations in 1993 rising to 398 in 1998.[6] The threat of untreatable multidrug-resistant bacteria has prompted a special report from the House of Lords[7] and a report on hospital-acquired infections by the National Audit Office.[8] The latter estimates that hospital-acquired infections and treatment of drug-resistant bacteria in the clinical setting cost the tax payer an estimated one billion pounds per annum.

The occurrence of a fully vancomycin resistant strain of MRSA in the US in 2002[9,10] indicates that the successful treatment of MRSA strains by the use of this glycopeptide antibiotic is not guaranteed. Linezolid (Zyvox®), a new member of the oxazolidinone group and the streptogramin quinupristin/dalfopristin mixture (Synercid®) are the newest anti-staphylococcal agents and have been heralded as a solution to MRSA infections. However, an isolated report of resistance to linezolid[11] in a clinical isolate of *Staphylococcus aureus* demonstrates that researchers should not be complacent in this area, and that there is a continual need for a pipeline of new agents to combat multidrug-resistant bacteria.

Abbreviations used in the review include SA (*Staphylococcus aureus*), SE (*Staphylococcus epidermidis*), MRSA (methicillin-resistant *Staphylococcus aureus*), MDR (multidrug-resistance) and MIC (minimum inhibitory concentration),

2 Resistance modifying agents (RMAs)

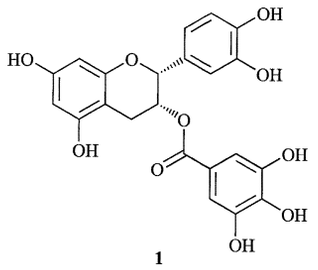
The concept of a compound that inhibits resistance in a bacterium which may be employed with a conventional antibiotic is well accepted and Augmentin® is an important example. This product, produced by GlaxoSmithKline, uses a combination of amoxicillin (a beta lactam antibiotic) and clavulanic acid, a microbially derived inhibitor of beta lactamases. The inhibitor greatly increases the stability of amoxicillin to degradation by beta lactamases[12] and the product is indicated for the treatment of patients with community-acquired pneumonia or acute bacterial sinusitis due to β-lactamase-producing pathogens.

Resistance modifying agents may also target and inhibit multidrug resistance (MDR) mechanisms. These are membrane proteins of varying substrate specificity which efflux antibiotics from the bacterial cell, resulting in a low intracellular ineffective concentration of the drug.[13] In combination with an antibiotic that is a substrate for these mechanisms, an inhibitor will increase the cellular concentration of the antibiotic therefore restoring its efficacy. It has also been shown that the use of such resistance modifying agents can also reduce the emergence of antibiotic resistant variants.[14]

2.1 Methicillin-resistance reversing agents

From the highly prolific group of Hamilton-Miller, much work on the ability of green tea and its constituents to inhibit methicillin-resistance in MRSA has been undertaken.[15,16] A patent[17] detailing the ability of 'compound P', which is also antibacterial (MIC = 280 μg ml^{-1}), to reverse methicillin-resistance has been described and the structure of this was revealed[18] as epicatechin gallate (**1**). This component appears to act by inhibiting the synthesis of penicillin binding protein 2′ (PBP2′) and is selective affecting only staphylococci that synthesise PBP2′. In the presence of a β-lactam antibiotic, epicatechin gallate renders MRSA strains sensitive, and an electron microscopy study has shown that **1** affects cell wall morphology of resistant strains whereas sensitive strains are unaffected. This highlights the potential of inhibiting the synthesis of PBP2′.

Diterpenes such as totarol (**2**) have also been shown to potentiate methicillin activity against MRSA *via* interference of PBP2′ expression.[19] When incorporated into the medium at 1 μg ml^{-1}, totarol caused at least an eight-fold increase in methicillin activity against an MRSA strain and totarol was antibacterial (MIC = 2 μg ml^{-1}) and inhibited respiration in *S. aureus*.



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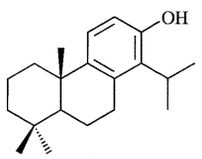

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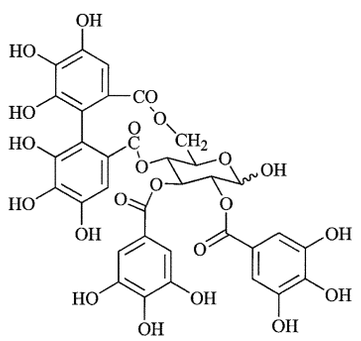
Two papers[20,21] from the Tsuchiya group on the effects of the hydrolysable tannins tellimagrandin I (**3**) and corilagin (**4**) show that these compounds work synergistically with oxacillin and that **4** reduces the MICs of various β-lactams but not other antimicrobial agents such as vancomycin, the fluoroquinolone ofloxacin or the macrolide erythromycin. The effects were seen against MRSA strains but not a methicillin-sensitive *Staphylococcus aureus* (MSSA) strain.[20] The authors suggest that the major action of this natural product is also by the inhibition of PBP2′ activity. In the presence of tellimagrandin I (**3**), the MICs of tetracycline against some strains of MRSA were also significantly reduced.[20]



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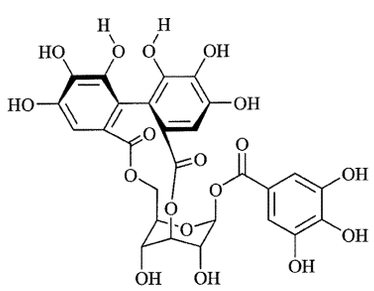

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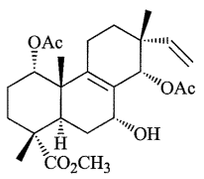
2.2 Modulators of multidrug resistance (MDR)

Some isopimarane diterpenes from *Lycopus europaeus* (Lamiaceae), typically **5**, have been shown to reduce the MICs of tetracycline and erythromycin by two-fold against strains possessing the Tet(K) and Msr(A) transporters which confer a high level of resistance to these antibiotics.[22] The MDR inhibitor reserpine (**6**) caused a four-fold reduction in the MIC of tetracycline although erythromycin activity was unaffected. The effects of (**6**) have been studied against a variety of multidrug-resistant MRSA and MSSA strains[23,24] and this compound

enhances the activity of tetracycline and norfloxacin against strains which possess efflux mechanisms such as the more specific Tet(K) protein and the MDR transporter NorA, the major drug efflux pump in this pathogen.[25] The NorA pump contributes significantly to decreased fluoroquinolone susceptibility. In one of these studies, reserpine reduced sparfloxacin, moxifloxacin and ciprofloxacin MICs up to four-fold in 11, 21 and 48 of 102 clinical isolates tested respectively.[24]



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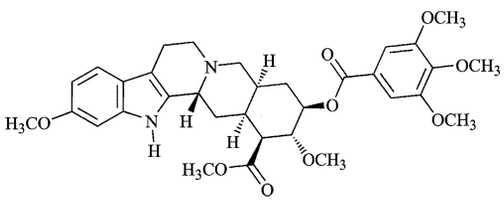

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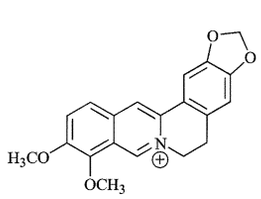
From the prolific groups of Stermitz and Lewis, a number of inhibitors of MDR in *S. aureus* have been described.[26–32] These workers studied the synergistic interaction between berberine (**7**), a plant antibacterial quaternary alkaloid produced by *Berberis fremontii* and another natural product, 5′-methoxyhydnocarpin (**8**) also present in this species.[26,27] Compound **8** potentiates the activity of berberine against strains possessing the NorA MDR transporter, and MDR-dependent efflux of berberine from cells was completely inhibited by this natural product. The authors postulate that the plant has evolved MDR inhibitors against MDR pumps in plant pathogens, and that in combination with latent antibacterial products (*e.g.* berberine), this offers an improved chemical defence. The flavonolignan silybin from the Milk thistle (*Silybum marianum*) of similar structure to **8**, and the porphyrin phaeophorbide A (**9**) (also from *Berberis fremontii*), have also been shown to be inhibitors of *S. aureus* MDR.[28,29] A key feature of these and many other MDR inhibitors is their large size and high degree of lipophilicity. These qualities are likely to be of importance for their solubility in the bacterial membrane and binding to the efflux transporters before inhibition can occur.



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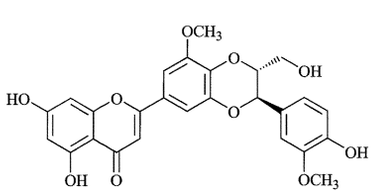

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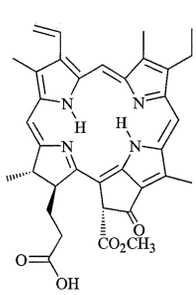

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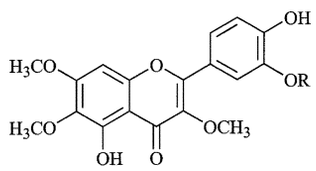
A number of methoxylated flavones[30] (**10**, **11**) and isoflavones[31] (**12**–**14**) that potentiate the activities of berberine and the synthetic fluoroquinolone antibiotic norfloxacin have been described. The first two flavones from Wormwood, *Artemisia annua* (Asteraceae) were earlier reported to potentiate the activity of the antimalarial artemisinin against the causative agent *Plasmodium falciparum,* and it is likely that in *S. aureus* (and possibly *P. falciparum*), these compounds exert their effects by inhibition of MDR pumps. Investigation of the isoflavones **12**–**14** from *Lupinus argenteus* has shown that they act as potentiators of berberine and linolenic acid antibacterial activity, the latter being present in this *Lupinus* species.



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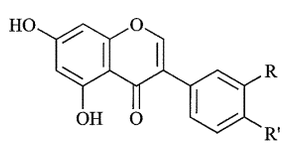
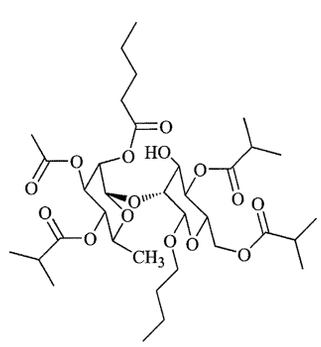
11 R = CH_3



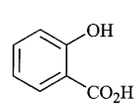
12 R = H, R' = OH
13 R = R' = OH
14 R = H, R' = OMe

MDR pump inhibitors have even been described from popular horticultural taxa such as *Geranium* with some polyacylated neohesperidosides from *G. caespitosum*[32] for example **15**, showing potentiation activity of berberine by increasing berberine uptake by inhibition of MDR. Compound **15** was also shown to be only weakly cytotoxic against three leukaemia cell lines.



15

Salicylic acid (**16**), present in many plant species, has recently been shown to induce a reduction in the accumulation of the fluoroquinolone antibiotic ciprofloxacin and the MDR substrate ethidium.[33] Inactivation of NorA, the major MDR transporter in *S. aureus*, did not alter the ability of salicylate to induce increased ciprofloxacin and ethidium resistance.



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3 Antibacterial natural products

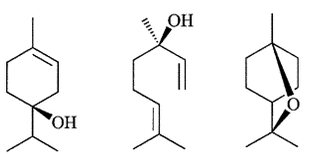
This section covers compounds which are directly antibacterial (cidal or static) and the section is broken down according to a general biogenetic source and an attempt has been made to

collate compounds according to structural similarity so that antibacterial activities can be compared according to class. Only the most active natural products from a literature source are mentioned and where mode of action is known this has been cited.

3.1 Monoterpenes

The tea tree (*Melaleuca alternifolia*, Myrtaceae) has long been regarded as a useful topical antiseptic agent in Australia[34] and there has been much research into the use of this oil as an antiseptic for nursing staff. The most active purified compounds from this oil include γ-terpinene, α-terpineol, terpinen-4-ol (**17**) and linalool (**18**) with MICs in the range of 0.125–0.25% v/v.[35–37] The most broad-spectrum of these being (**17**) with activity against Gram-negative bacteria.[36] It was also shown that non-oxygenated monoterpenes *e.g.* γ-terpinene and *p*-cymene reduce the efficacy of **17** by reducing its aqueous solubility.[37]

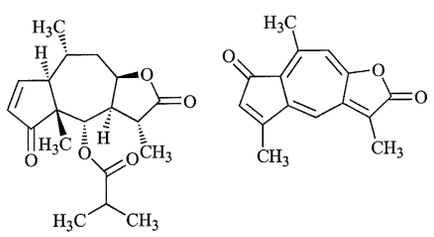
From *Artemisia asiatica*,[38] 1,8-cineole (**19**) was found to be the major anti-staphylococcal agent of the essential oil with an MIC of 2 μl ml^{-1}. This was also active against the Gram-negative species *Escherichia coli* and *Pseudomonas aeruginosa* (both MICs of 3 μl ml^{-1}) which in many cases are insensitive to plant antibacterials unless high concentrations are employed.



17 **18** **19**

3.2 Sesquiterpenes

In a study to ascertain the active principles of a Nepalese medicinal plant used to treat sinus infections,[39] the guaianolide sesquiterpene lactone 6-*O*-isobutyroplenolin (arnicolide C) (**20**) was characterised as the most active against a methicillin-sensitive *Staphylococcus aureus* (MSSA) strain (MIC 38 μg ml^{-1}). The authors postulate that the activity depends on the presence of a *beta* unsubstituted cyclopentenone ring moiety and work by others[40] showed that saturation of this dramatically reduced activity. Interestingly, the antibacterial activity appears to be independent of an α-methylene-γ-lactone moiety, although this group is needed for significant antitumour activity. Furthermore, compound **20** was shown to be bactericidal and not bacteriostatic as bacteria grown in the presence of this product could not be recultured. The related guaianolide **21**, from *Artemisia gilvescens*[41] showed excellent potential against a clinical strain of MRSA (MIC, 1.95 μg ml^{-1}) and only moderate cytotoxicity toward a human colon carcinoma cell line



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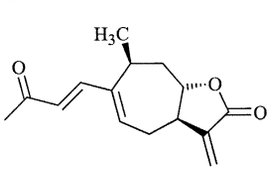

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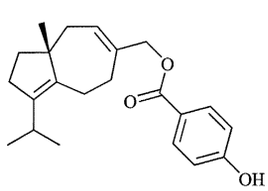
(IC_{50} = 16 μM) indicating that possibly this margin can be exploited. Xanthatin (**22**), exhibited species specific activity with only Gram-positive bacteria being affected and the authors profiled **22** against twenty MRSA and seven MSSA with MIC values being comparable for resistant and sensitive strains.[42]

Daucane sesquiterpenes with various aromatic ester moieties attached to them have been characterised from *Ferula* species, notably *F. hermonis*[43] (**23**, MIC, 6.25 μg ml^{-1}) and *F. kuhistanica* (**24**, MIC, 8–16 μg ml^{-1}).[44]



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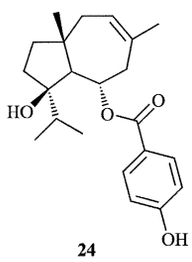

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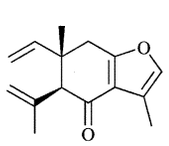
Sesquiterpene ester **24** was hydrolysed and the antibacterial activity of the hydrolysis products investigated. The sesquiterpene portion had greatly reduced activity (MIC, 125 μg ml^{-1}) and *p*-hydroxybenzoic acid was inactive indicating the importance of both moieties for overall activity.[44]

Much work has been conducted on myrrh (*Commiphora molmol*) antimicrobial properties[45] as these exudates are produced by trees belonging to the Burseraceae family, which secrete resins rich in terpenoids and carbohydrates that are probably produced by the tree as a defence against microbial and insect attack following damage. The furano *seco*-A-ring sesquiterpene curzerenone **25** was isolated along with other sesquiterpene mixtures as being responsible for the anti-staphylococcal activity (MIC = 0.7 μg ml^{-1}) and this compares very well with ciprofloxacin activity against the same strain (MIC = 0.12 μg ml^{-1}).[45] This may account for the use of myrrh in antiquity for treating wounds and as a local eye medication.[46]

Mansinone F (**26**) from *Ulmus davidiana* var. *japonica* exhibited superb activity *versus* 19 MRSA strains with MIC values in the range of 0.39–3.13 μg ml^{-1} and these compare favourably with vancomycin (0.39–1.56 μg ml^{-1}), the most widely used anti-MRSA antibiotic.[47] The authors mention that this structurally simple and unique *ortho*-naphthoquinone offers much potential as a new anti-MRSA lead and certainly this compound is amenable to synthetic modification and has potential as a template for analogue synthesis.

The aromatic sesquiterpene phenol, xanthorrizol (**27**),[48,49] had activity against SA and MRSA in the range of 16–32 μg ml^{-1} and this compound was shown to non-specifically inhibit DNA, RNA and protein synthesis[49] but a single oral dose did not protect mice infected with SA introduced intraperitoneally.



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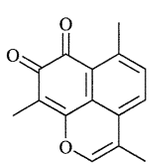

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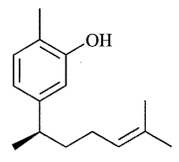

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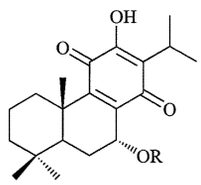
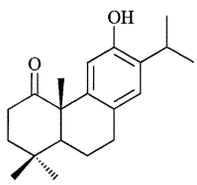
3.3 Diterpenes

This is one of the largest groups of plant derived natural products with anti-staphylococcal activity and certain plant taxa and diterpene classes are well represented. In particular, the genus *Salvia* from the Lamiaceae or mint plant family, is prolific and a recent important review covers the antibacterial and cardioactive properties of these species.[50]

From *Salvia blepharochlaena*,[51] the abietane diterpenes horminone (**28**) and 7-acetylhorminone (**29**) were active toward SA and *S. epidermidis* in the MIC range of 1.5–10 μg ml^{-1} and a similar level of activity is seen from 1-oxoferruginol (**30**) from *Salvia viridis*.[52]



28 R = H
29 R = Ac



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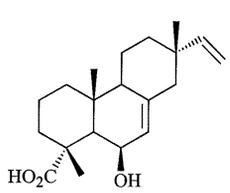
Salvia additionally produces isopimarane type diterpenes[53] *e.g.* **31** having comparable activity (9 μg ml^{-1})[54] to amikacin (16 μg ml^{-1}), ampicillin (8 μg ml^{-1}) and cefoperazone (16 μg ml^{-1}) which are commonly used to treat infections caused by Gram-positive bacteria. Abietane quinones from *Salvia prionitis*[55] and *S. lanigera*[56] (*e.g.* sanigerone, **32** MIC = 13 μg ml^{-1} against SA) highlight the potential of these compounds as lead structures. Sanigerone (**32**) is a *nor*-diterpene, presumably formed *via* oxidation of methyl-20 and decarboxylation resulting in the formation of an unusual aromatic B-ring abietane. Other taxa in the mint family excel at producing anti-staphylococcal compounds and these include *Plectranthus hereroensis*, which produces an acetylated abietane quinone (**33**, MIC = 31.2 μg ml^{-1})[57] related to horminone (**28**), and *P. elegans*[58] (**34** and **35**) with similar activities against Gram-positive bacteria, and causing inhibition of fungal spore germination (*Cladosporium cucumerinum*). The authors suggest that these compounds may have a role in the chemical defence of *Plectranthus.*[58]



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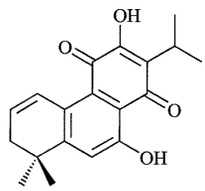

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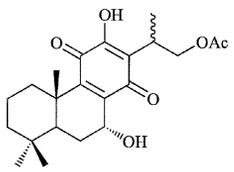

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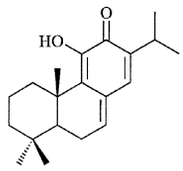

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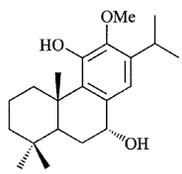
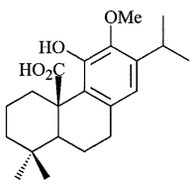


35

From *Dauphinia brevilabra* (Lamiaceae) the methoxylated abietic acid (**36**)[59] showed good potency of 1 μg ml^{-1} against a standard laboratory SA strain (NCTC 6751) with the same activity as chloramphenicol. Other reports of lamiaceous species producing antibacterial diterpenes include *Ballota saxatilis* subsp. *saxatilis* which yielded the furano-labdane diterpene ballonigrine (**37**) with moderate activity against SA and *S. faecalis* (25 μg ml^{-1}).[60]



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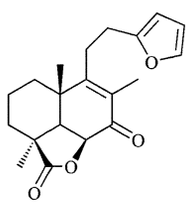
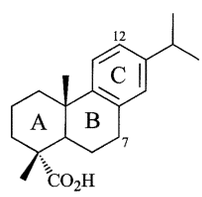


37

Coniferous plants (Pinopsida) are an interesting source of antibacterial leads, again being rich in abietane type diterpenes from *Cephalotaxus*[61] and *Chamaecyparis*[62] species, with MIC values below 15 μg ml^{-1} and there is potential to exploit this taxonomic group and the abietane diterpene class[63] providing that adequate dereplication can be carried out to avoid common and active natural products such as abietic acid itself.

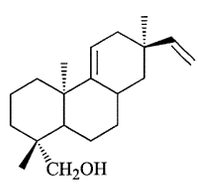
The dehydroabietic acid (**38**) skeleton has been extensively investigated to determine structural effects on anti-staphylococcal and antifungal activity.[64] This study showed that simple derivatisation of dehydroabietic acid, for example, conversion of the acid to aldehyde or alcohol, improved activity and that removal of the isopropyl side chain from ring-C or the introduction of an alcohol or ketone at C-7 or C-12 could enhance activity depending on the organism. The stereochemistry of the A/B ring junction did not appear to display a significant role in antibacterial activity.



38

An excellent study has recently been conducted by Timmermann and co-workers[65] on the isopimarane **39**, using SA and MRSA strains and activity was also shown against *Bacillus subtilis* (MIC; 2, 2 and 4 μg ml^{-1} respectively). These authors showed that the presence of an oxymethylene group at C-19 is important to activity, since its replacement with a carboxylic acid reduces activity. This is mirrored in the above case of abietane diterpenes. Compound **39** rapidly and non-specifically inhibited uptake and incorporation of radio-labelled thymidine, uridine and amino acids at its MIC. The authors suggest that this may function by a membrane damaging effect although no specific mechanism is described. Using human red blood cells however, no haemolytic effect was observed until a concentration of 32 μg ml^{-1} was used. The compound was also evaluated *in vivo* for its ability, at a subcutaneous dose, to afford protection against SA infection but no protection was seen in a murine model.[65]

Work has been conducted on the totarane diterpene totarol (**40**) during the period of this review to ascertain mode of action[66] and structure activity relationships of derivatives.[67] This product originally comes from a coniferous plant *Podocarpus nagi,* and has activity against MRSA[68] and its activity is improved when tested in combination with other natural products.[69] The Kubo group[66] have demonstrated that **40** inhibits oxygen consumption and respiratory-driven proton translocation in whole cells, and oxidation of NADH in a membrane preparation. Amongst several key enzymes studied, NADH-cytochrome *c* reductase was inhibited whilst cytochrome *c* oxidase was not. The authors postulate that the site of respiratory inhibition of totarol was near CoQ in the bacterial electron transport chain.



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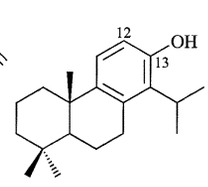
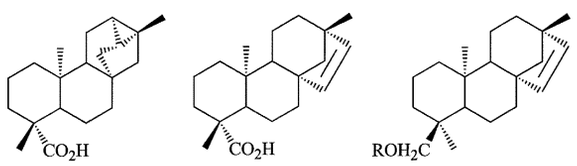


40

Modifications at C-12 and O-13 of totarol on antibacterial properties have been evaluated and in general a phenolic moiety

is essential for activity of <32 μg ml^{-1}. Derivatisation of C-12 was deleterious and totarol and its derivatives appear to be only active against Gram-positive species.[67]

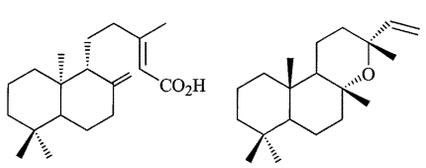
Some of the rarer skeleta of diterpenes include the trachylobane class, typically **41** which has activity (both at 6.25 μg ml^{-1}) against SA and *Mycobacterium smegmatis,* a model for assessment of anti-tubercular drugs.[70] Again this compound is oxygenated at one of the methyl groups at C-4, which seems to be a regular feature of many antibacterial diterpenes. This is the case with beyerenoic acid (**42**) from the roots of *Viguiera hypargyrea* (Asteraceae) with activity toward SA and *Enterococcus faecalis* (MIC; 12 μg ml^{-1}) and this supports the use of these roots as a treatment of gastrointestinal disorders in Mexico.[71] *Fabiana densa* var. *ramulosa* is used in traditional medicine in Chile to treat coughs and illnesses of the lungs, and a bioassay-guided study led to the isolation of the succinate derivative **43** (MIC < 10 μg ml^{-1}),[72] which is structurally related to **42**.



41 **42** **43** R = succinate

Continuing in the same theme of South American medicinal plants yielding useful anti-staphylococcal leads, Tincusi *et al.*, have investigated an oleoresin from Peruvian *Copaifera paupera* (Leguminosae). Diterpene **44** was active against SA and *S. epidermidis* at 5 and 10 μg ml^{-1} respectively and the control, cephotaxime had an MIC of 2.5 μg ml^{-1}.[73]

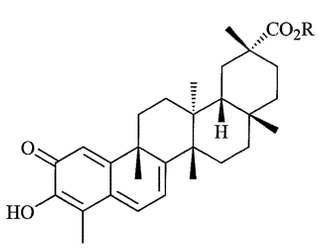
Investigation of essential oils of two *Helichrysum* species led to the characterisation of the cyclised labdane diterpene manoyl oxide (**45**) as having bacteriostatic activity against SA (MIC = 50 μg ml^{-1}).[74]



44 **45**

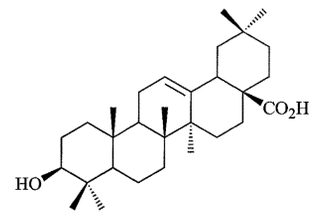
3.4 Triterpenes

All of the triterpenes reviewed are either acids or esters and the predominant skeleton is the Δ12-oleanene pentacycle. There are two *nor*-friedelane triterpenes (**46** and **47**) from *Crossopetalum gaumeri* with excellent potency toward *S. epidermidis* (0.54 and 1.11 μM respectively) when compared to chloramphenicol (12.4 μM).[75] These compounds are presumably formed *via* by decarboxylation of methyl-24 allowing unsaturation in rings A and B.



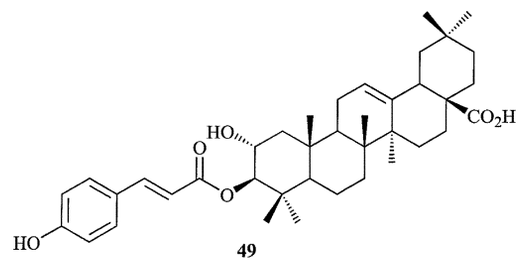
46 R = H
47 R = Me

Even simple compounds that are almost phytochemically ubiquitous such oleanolic acid (**48**) show appreciable anti-staphylococcal activity (MIC 8 and 16 μg ml^{-1}) against SA and



48

MRSA, and this compound was isolated from fractions with higher activity that contained polyphenolic components.[76] Esters of this skeleton, notably at the C-3 position such as **49** possessing a *trans-p*-coumarate, have been isolated and tested against SA and *Staphylococcus capitis* with 12.5 μg ml^{-1} MIC values[77] and even simple 29-oic acid derivatives possessing hydroxyl groups around the skeleton have similar activity such as maytenfolic acid (**50**).[78]



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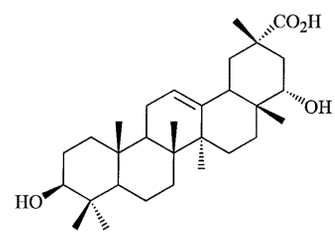

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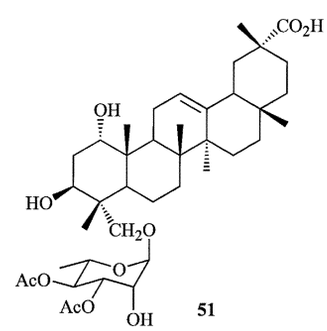
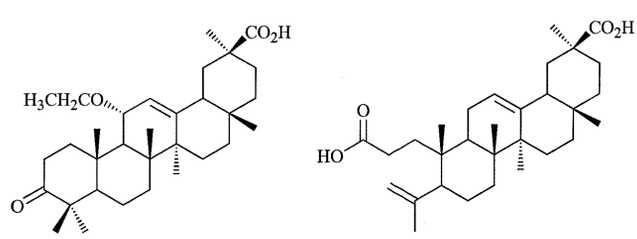
Triterpene glycosides are also represented and the acetylated rhamnoside oleanene (**51**)[79] and its corresponding aglycone have excellent potency *versus* SA with MICs at 6.25 and 3.13 compared to streptomycin at 0.78 μg ml^{-1}.



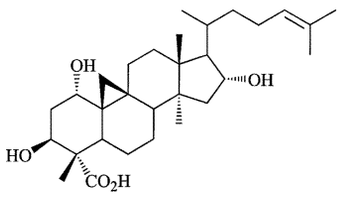
51

Muhammad *et al.*,[80] report an 11-ethoxy derivative (**52**) and a *seco*-A-ring oleanene, koetjapic acid (**53**), from *Maytenus undata* (Celastraceae) (MIC; 50 and 12.5 μg ml^{-1}; SA) and the *seco* compound is probably related to other oleananes present



52 **53**

in the plant *via* a Baeyer–Villiger type oxidation. The prolific group of Timmermann and co-workers, have characterised from *Acalypha communis* (Euphorbiaceae), several active cycloartane type triterpenes[81] with **54** demonstrating activity against SA, MRSA and a vancomycin-resistant *Enterococcus faecium* at 32, 64 and 8 μg ml^{-1} compared to penicillin G at 0.06, 128 and 128 μg ml^{-1} respectively.



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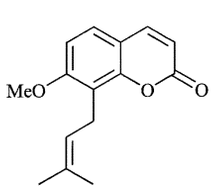
3.5 Phenylpropanoids and stilbenoids

In this section, natural products with the structural features of C_6–C_3 and C_6–C_2 moieties are covered and this includes the phenylpropanoids, coumarins, lignans and stilbene related compounds. Coumarins are known to have extensive anti-bacterial activity and there are microbially derived examples used clinically *e.g.* novobiocin, and these agents are thought to exert their effects by inhibition of bacterial DNA gyrase.[82] Osthol (**55**) from *Prangos pabularia*[83] is a 7-methoxylated, 8-prenylcoumarin with an MIC of 31.25 μg ml^{-1} towards a methicillin-resistant strain but was inactive against *E. coli* and *P. aeruginosa*, which is presumably due to poor penetration through the cell wall of the Gram-negative species. Compound **56**, bornyl coumarate, of mixed biosynthesis possessing both phenylpropanoid and monoterpene moieties, had excellent activity against a standard SA strain (0.6 μg ml^{-1}).[84] Presumably, the lipophilic monoterpene portion of the molecule allows membrane permeability of this compound and the phenolic coumarate may act as an ionophoric moiety. The *nor*-lignan (**57**)[85] from *Styrax ferrugineus* (Styracaceae), a plant which is used in Brazil to treat wound infections, was isolated with a series of other *nor*-lignans, some of which are glycosides, has an MIC of 10 μg ml^{-1} compared to chloramphenicol (SA, 5 μg ml^{-1}). This is a simple achiral metabolite and would be an excellent starting template to synthesise analogues to enhance potency.



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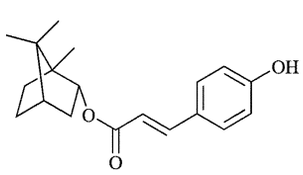

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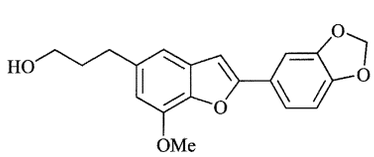

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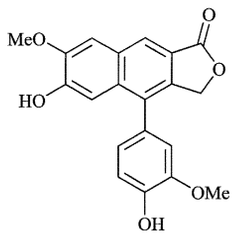
Other relatively simple examples include full lignans of the aryl tetralin class *e.g.* **58** and **59**, which are biosynthetically related to the cytotoxic podophyllotoxin group.[86] Both of these compounds were profiled using 18 strains of MRSA with values ranging from 4–32 μg ml^{-1} and interestingly these compounds are only active toward MRSA strains and not MSSA (methicillin-sensitive SA). This selectivity is intriguing and the authors are investigating the mechanism of how these agents



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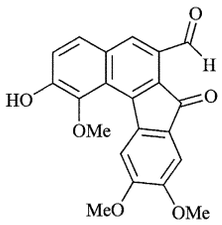

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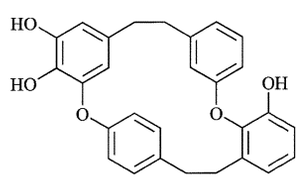
function. This highlights the value of screening metabolites against resistant and sensitive strains, particularly isolates of direct clinical relevance.

Marchantin A (**60**) from a Hungarian liverwort *Marchantia polymorpha*[87] has exceptional activity against a panel of both Gram-positive and Gram-negative bacteria (SA; MIC = 6.8 nM (!)) and as this compound is cyclic and has lipophilic and hydrophilic domains, it is possible that it functions by forming pores in cell membranes resulting in cell lysis. The exceptionally complex vaticaphenol A (**61**) (not a tempting synthetic target), is a resveratrol tetramer and was isolated employing bioassay-guided fractionation of extracts of the stem bark of *Vatica oblongifolia* ssp. *oblongifolia* (Dipterocarpaceae) from Sarawak.[88] This metabolite has moderate activity toward SA and *Mycobacterium smegmatis* (50 and 25 μg ml^{-1} respectively) but shows the best possible features of plant derived natural products being highly functional and chiral. The productive research group of Ilias Muhammad have isolated and characterised from *Machaerium multiflorum,* a series of highly unusual and rare stilbenoid-monoterpenes (*e.g.* **62**).[89,90] These agents are hexahydrobenzopyrans and these are the first reports of this type of compound from a higher plant other than the genus *Cannabis*, and **62** was active against SA and MRSA (5 and 4.5 μg ml^{-1} respectively).



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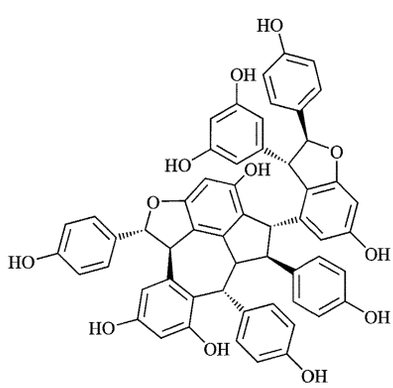

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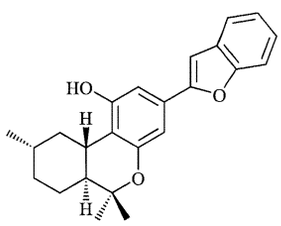
3.6 Simple phenols and tropolones

The leaves of *Piper gibbilimbum* (Piperaceae) is a scrambling shrub used in Papua New Guinea as an antiseptic to heal abscesses, ulceration of the skin and also to treat fevers. Fractionation of the petroleum extract of the leaves of this plant afforded several alkenylphenols (*e.g.* gibbilimbol B; **63**,

Table 1 Flavonoid anti-staphylococcal natural products

Compound	Subclass	Bacterium	MIC[a]	Compound	Subclass	Bacterium	MIC[a]
69	Chalcone[96,97]	SA	3.0	**78**	Flavanone-stilbene[107]	MRSA	3.13
70	Dihydrochalcone[98]	SA/MRSA	10/4.5	**79**	Isoflavanone[108]	SA	8.3
71	Flavone[99]	MRSA	3.9–15.6	**80**	Isoflav-2-one-4-ol (3-phenylcoumarin)[109]	SA	9.7
72	Flavone[99]	MRSA	62.5–125	**81**	Isoflavan[110]	MRSA	3.13
73	Flavanone[100]	SA	50	**82**	Isoflavan[97]	SA/MRSA	3.13/6.25
74	Flavanone[101]	SA/MRSA	1.56/1.56	**83**	Pterocarpan[111]	MRSA	3.13–6.25
75	Flavanone[102,103]	SA/MRSA	3.13–6.25	**84**	Pterocarpan[111]	MRSA	3.13–6.25
76	Flavanone[105]	SA/SE	5.0/5.0	**85**	Dimer[113]	SA	15.3
77	Flavanone[106]	SA/SE	1.8/1.8	**86**	Dimer[113]	MDR/MRSA	8

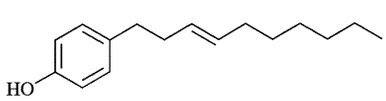
[a] In μg ml^{-1}; SA = *Staphylococcus aureus*; MRSA = methicillin-resistant *Staphylococcus aureus*; SE = *Staphylococcus epidermidis*; MDR = multidrug-resistant strain.



62

MIC = 2 μg ml^{-1}, SE) which were evaluated using Brine Shrimp and KB nasopharyngeal carcinoma cells (ED_{50} (**63**) 3.9 μg ml^{-1}).[91]

A semisynthetic study on derivatives of alkanin (**64**), which has been esterified by a range of alkyl substituents at position C-1′ was conducted employing vancomycin-resistant enterococci and MRSA.[92] The parent natural product (**64**) exhibited MIC values of 6.25 μg ml^{-1} against MSSA and MRSA but the semisynthetic small branched alkyl esters had greater potency (with the exception of aromatic esters).



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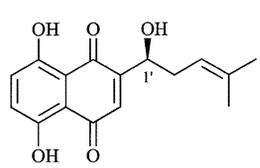

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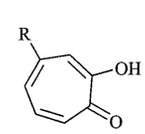
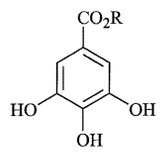
Tropolones such as 4-acetyltropolone (**65**) and hinokitiol (**66**) have been shown to have very low MICs against *S. epidermidis* (1.56 and 0.2 μg ml^{-1} respectively) and activity is suggested as being attributable to metal chelation between the carbonyl group at C-1 and hydroxyl at C-2 in both molecules.[93] Even simple acids and esters such as gallic acid (**67**) and ethyl gallate (**68**) have been evaluated for their anti-MRSA properties with MIC values recorded against 20 strains of MRSA and 7 strains of MSSA.[94] These compounds exhibited no activity toward *S. epidermidis* (MIC > 1000 μg ml^{-1}) and MIC values toward SA ranged from 15.7 to 62.5 μg ml^{-1}. The authors were prompted to investigate these natural products by work carried out by Kono *et al.*,[95] on the antimicrobial activity



65 R = CHO
66 R = isopropyl



67 R = H
68 R = ethyl

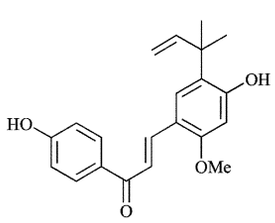
of epicatechin gallate (**1**) against clinical isolates of MRSA, because epicatechin gallate can produce gallic acid through its hydrolysis.

3.7 Flavonoids

The activities of this group, which is one of the largest anti-staphylococcal (and broadly antibacterial) classes of metabolite, are reported in Table 1 where the subclass of flavonoid is also detailed.

Chalcone[96,97] and dihydrochalcone[98] flavonoids are represented by **69** and **70** and these, probably the simplest of flavonoids, have very respectable activities. The simplicity of these structures and the ease with which combinatorial libraries could be prepared from this template make them an attractive target, particularly licochalcone A (**69**) (originally isolated from liquorice), which would be amenable to a variety of prenyl and alkyl substitutions.

Even simple flavones[99] such as apigenin (**71**) and luteolin (**72**) exhibit good to moderate activities against SA and MRSA strains (3.9–62.5 μg ml^{-1}), and these compare with methicillin MIC values which, in some cases, were 1000 μg ml^{-1} against the same resistant strains.



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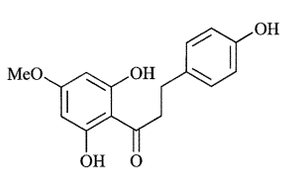

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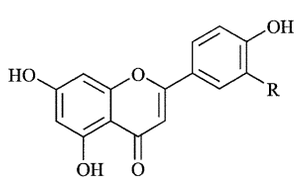
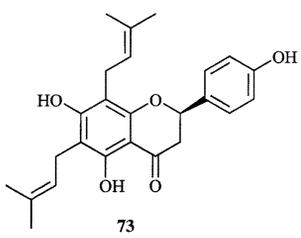


71 R = H
72 R = OH

It is the flavanones which are the most widely reported anti-staphylococcal flavonoids[100–106] and all of those reported here (**73**–**77**) possess either prenyl (sometimes more than one) or geranyl groups that presumably contribute to the lipophilicity and membrane solubility of these compounds. Of particular note[101] within this group is **74** with excellent potency toward standard and MRSA strains (Table 1). Sophoraflavanone G (**75**) is antibacterial[102] but also has strong synergism[103] in combination with vancomycin with a fractional inhibitory concentration (FIC) index of 0.16. This is a significant effect as FIC indices are an indicator of synergistic effects where FIC <0.5 relates to synergism.[104] This effect was also seen to a lesser extent with other antibiotics and the authors propose that a combination of **75** with vancomycin may contribute to better treatment of an MRSA infection. Flavanone **77** is related to sophoraflavanone G and isomeric with respect to the geranyl (lavandulyl) subunit[106] and an investigation on the impact of these monoterpene side chains on potency is valuable. Metabolite **78**, alopecurone B, is a flavanone-stilbene,[107] which when tested against 21 strains of MRSA, had potencies of between 3.13 and 6.25 μg ml^{-1} that compare highly favourably with methicillin (12.5–>100 μg ml^{-1}), gentamicin and erythromycin (both in the range 1.56–>100 μg ml^{-1}).



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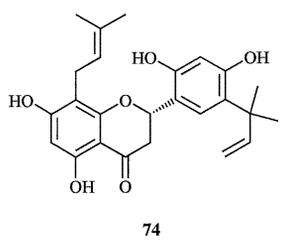

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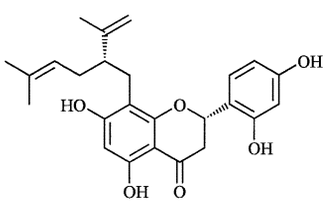

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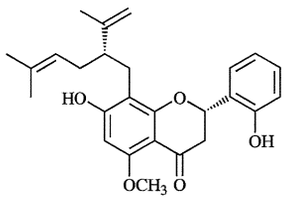
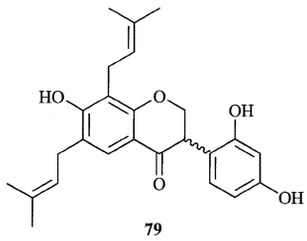


76

The genus *Erythrina* (Fabaceae) produces a number of prenylated isoflavonoids,[108–110] (*e.g.* **79**–**81**) and these are also present in *Glycyrrhiza* species[97] of the same family (*e.g.* **82**). Compound **80** is also a 3-phenylcoumarin and it has been postulated that these compounds exert their effects by inhibition of bacterial DNA gyrase.[82]

The pterocarpans (*e.g.* **83** and **84**), which are biosynthetically related to isoflavonoids and have similar anti-staphylococcal properties, occur within the same genera and species *e.g. Erythrina zeyheri*.[111] These metabolites are known to be phytoalexins which are antimicrobial natural products biosynthesised *de novo* following colonisation of plants by bacteria and fungi.[112] This ecological rationale for the presence of antibacterial products should certainly be exploited in lead discovery.

In Botswana and Zimbabwe, *Vahlia capensis* is widely used to treat eye infections and Majinda *et al.*,[113] have isolated an



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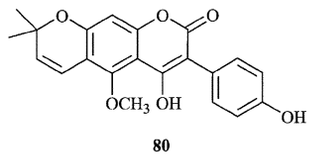

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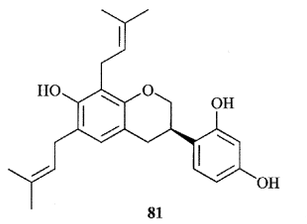

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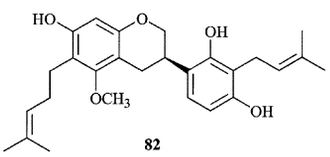

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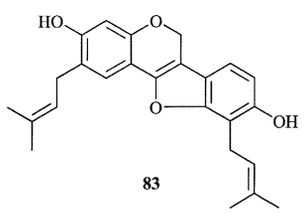

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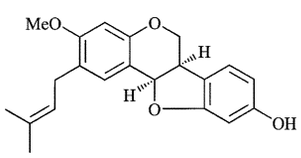

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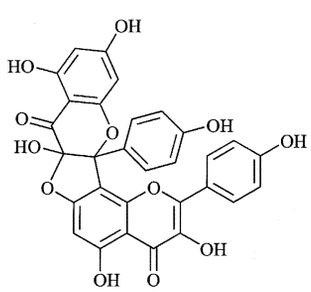
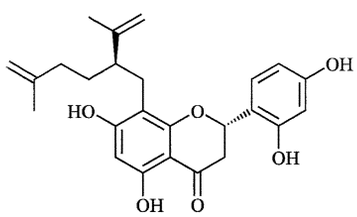


85

antibacterial flavonoid dimer (**85**) which may account for the traditional use of this species. The Washambaa people of the Western Usmabara Mountains of Tanzania use the yellow bark of *Ochna macrocalyx* for gastrointestinal disorders and from extracts of this species, a series of flavonoid dimers *e.g.* **86** have been isolated. This compound exhibited good activity (MICs = 8 μg ml^{-1})[114] against three SA strains which possess efflux mechanisms, one of which is the NorA MDR transporter, the major drug efflux pump in this species.

3.8 Alkaloids

There is excellent rationale that plant alkaloids should possess antibacterial activity, particularly given the number of cytotoxic drugs and templates from this source such as the vinca alkaloids (vincristine and vinblastine) and camptothecin and its synthetic derivatives (topotecan and irinotecan). From *Clausena heptaphylla* (Rutaceae), **87** has a broad spectrum of activity with MIC values of 3, 6 and 20 μg ml^{-1} against SA, *Escherichia coli* and *Pseudomonas aeruginosa*.[115] This activity toward the Gram-negative species, which are generally harder



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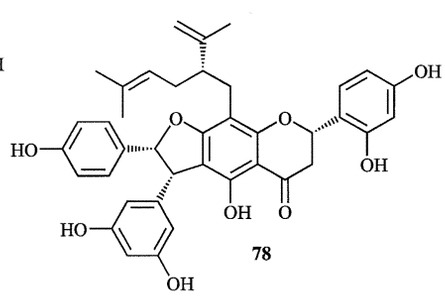

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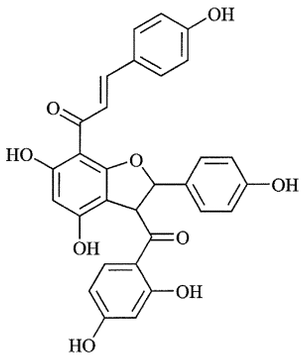
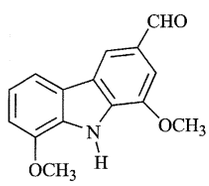


86

to find hits against, and the simple nature of this carbazole alkaloid is intriguing. From the same carbazole class, mahanine (**88**) showed a wide range of biological activities (MIC 12.5 μg ml^{-1}; SA), including cytotoxicity toward HL60 tumour cells (MIC_{100} = 4.0 μg ml^{-1}). Interestingly, this compound is also antimutagenic and in an Ames test was able to inhibit mutations caused by heterocyclic amines by 99% at a concentration of 20 μM, and no toxicity was seen against *Salmonella typhimurium* at this concentration.[116]



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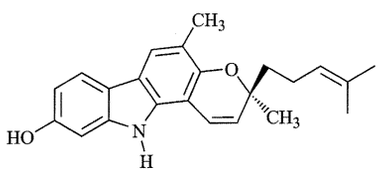
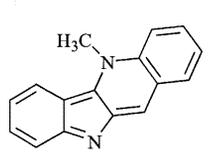


88

Cryptolepis sanguinolenta is widely used in West and Central Africa to treat infectious disease and the major active antimicrobial principle, cryptolepine (**89**) has been profiled using an extensive panel of Gram-negative, Gram-positive bacteria and yeasts. The MIC of the free base was less than 7.8 μg ml^{-1} whereas the hydrochloride salt was less active (60 μg ml^{-1}) toward *S. aureus.* This is unusual given the increased water solubility of the salt which may in fact be detrimental to cellular absorption (and hence reduced activity) of this indoloquinolizidine.[117] There are few examples of polymeric alkaloids in the literature and from *Calycodendron milnei* (Rubiaceae) a series of small polymers based on the pyrrolidinoindoline skeleton have been isolated.[118] One of these, isopsychotridine (**90**) (MIC; 5 μg ml^{-1}; SA), has five monomers joined together in which the first two monomer units are coupled through the alicyclic carbons C-3a to C-3a and further linkages are formed *via* bonds between the aromatic carbon C-7 and the C-3a carbon of another monomer. These agents also display antifungal, anti-yeast, anti-viral and cytotoxic activities.[118]



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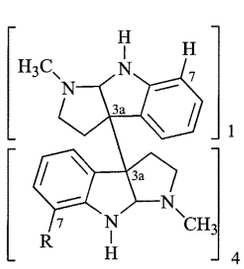
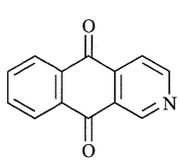


90

From the Clark group in Mississippi, an unusual azaanthraquinone (**91**) with an MIC of 6.25 μg ml^{-1} toward SA from *Mitracarpus scaber* (Rutaceae) has been characterised and compared with a commercially available synthetic sample.[119] Goldenseal (*Hydrastis canadensis*) is a widely used herbal product, especially in the United States for the treatment of a variety of gastric disorders.[120] It is a small perennial plant found in North American damp forests and is used by the Cherokee Indians as a disinfectant.[121] Extracts and isolated compounds from this species have been evaluated and the most active anti-staphylococcal compound is the quaternary ammonium alkaloid berberine (**7**) (MIC; 31 μg ml^{-1}; SA) which is present in the plant in high concentration (6%) and provides a rational basis for the traditional antibacterial use of Goldenseal.[122]

The Southern prickly ash (*Zanthoxylum clava-herculis,* Rutaceae), also produces quaternary alkaloids, and one of these chelerythrine (**92**), is the major anti-staphylococcal agent from this species. This agent has activity against a standard *S. aureus* ATCC 25923 strain (4 μg ml^{-1}) and toward three strains possessing the efflux mechanisms MsrA, TetK and NorA with MIC values of 8, 16 and 8 μg ml^{-1} respectively.[123] These activities compare well with the activities of erythromycin (64 μg ml^{-1}), tetracycline (256 μg ml^{-1}) and norfloxacin (32 μg ml^{-1}) against these resistant strains and indicate that chelerythrine may be a poorer substrate for these efflux systems than the antibiotics. Whilst chelerythrine in a known cytotoxin, it is possible that modification of the benzo[*c*]phenanthridine template could reduce the cytotoxicity and retain the antibacterial activity of this group toward effluxing strains.



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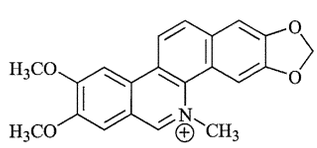
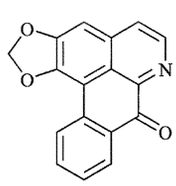


92

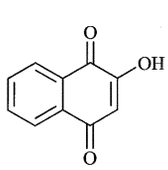
The final alkaloid to be reviewed is the aporphine **93** that comes from the annonaceous *Guatteria multivenia,* which has been evaluated against a series of yeasts (*C. albicans, Cryptococcus neoformans*) and SA and MRSA strains with MIC values of 2.0 μg ml^{-1} for the staphylococci.[124]



93

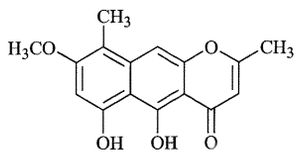
3.9 Polyketides and polyynes

An important paper published by Tegos *et al.*,[125] has shown that certain plant antimicrobials such as the polyketide derived rhein and plumbagin (**94**) have striking activity against MDR *S. aureus* and some Gram-negative bacteria when the MDR pumps within these species are disabled. The potentiation of activity is in some cases 100 to 2000-fold. This has great broad-spectrum potential. Furthermore, measurement of uptake of a plant metabolite (berberine) confirmed that disabling the MDRs strongly increased the level of penetration of the plant antimicrobial. The authors suggest that plants may have developed a way of delivering their antimicrobials into bacteria.



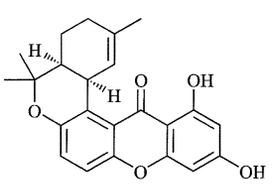
94

Again from the prolific research group of Alice M. Clark, the naphthopyrone **95**, from *Cassia quinquangulata*[126] has excellent potency toward SA and MRSA strains (MIC; 3.125 and 6.25 μg ml^{-1}) and the genus *Cassia* which includes the Senna species are prolific producers of polyketide derived naphtho- and anthraquinone phenolics and are certainly worth exploring further.



95

Xanthones (**96**–**99**) are metabolites commonly found in the Clusiaceae (Guttiferae) family and frequently occur as prenylated, geranylated or farnesylated products. Calozeyloxanthone, **96**, from *Calophyllum moonii* and *C. lankensis*[127] was profiled against 17 strains of SA (MIC; 4.1–8.1 μg ml^{-1}) comparing favourably with vancomycin (0.5–4 μg ml^{-1}) and this agent should certainly be further investigated using *in vivo* models. From another guttiferaceous plant, *Garcinia dioica,* rubraxanthone (**97**) has even better *in vitro* potency than vancomycin and is one of the most potent anti-MRSA agents from plants to date, having MIC values ranging from 0.313–1.25 μg ml^{-1} toward MRSA and MSSA strains.[128] The lipophilic nature of this compound is probably responsible for good bacterial uptake and the authors anticipate that these xanthones will have wide pharmaceutical uses. Further examples of this class include globulixanthones D and E (**98** and **99**)[129] (MIC; 8.0, 4.5 μg ml^{-1}; SA respectively) and the dimer (**99**) may well be a DNA intercalator having an interesting shape following rotation around C-5–C-8′ bond which may fit into a DNA groove.



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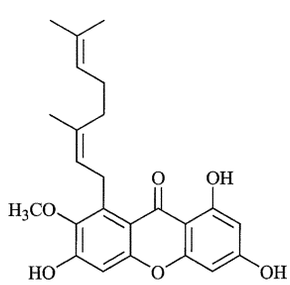

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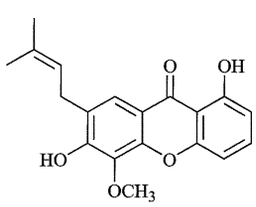
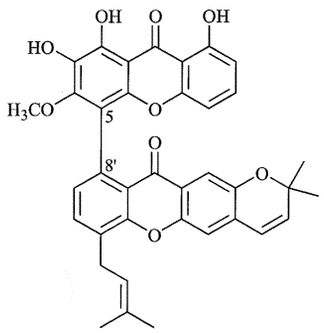


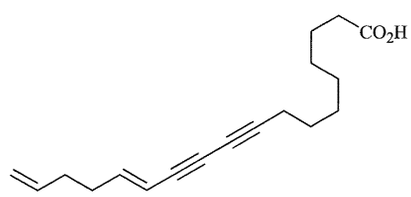
98

Simple C_6 compounds such as (3*E*)-hexenal display *S. aureus* bacteriostatic effects at low concentration (0.1–1 μg ml^{-1}),[130] and these compounds are part of the scent or 'green odour' of plant leaves and probably contribute to antimicrobial plant defence. Other straight-chain polyketides include the poly-



99

acetylenes which have wide distribution in several plant families (Asteraceae, Araliaceae and Apiaceae) and are in some cases produced as phytoalexins. The polyynes **100** and **101** from *Mitrephora celebica*[131] (Annonaceae) had moderate activity (MIC; 25 and 12.5 μg ml^{-1}, MRSA) and these acetylenic compounds are not commonly found in the Annonaceae. The authors remark that these acetylenic acids were unstable and decomposed to blue methanol insoluble products. Falcarindiol (**102**) is more stable, and two separate studies[132,133] one of which defined the absolute stereochemistry of this product using Mosher's ester methodology as 3(*R*),8(*S*),[133] have evaluated the anti-staphylococcal nature of this metabolite with activity toward three strains of MDR SA of between 8–16 μg ml^{-1} and epidemic MRSA strain-15 was also evaluated (MIC 32 μg ml^{-1}).



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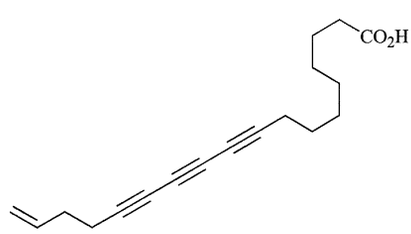

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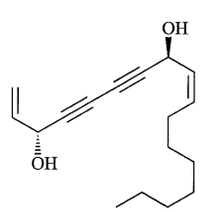
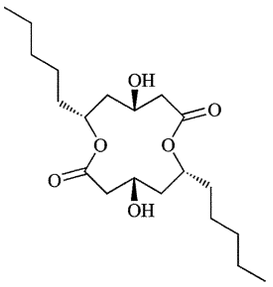


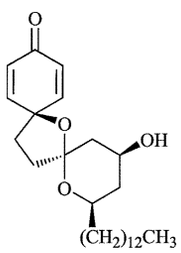
102

Verbalactone (**103**) from *Verbascum undulatum* is a cyclic lactone derived from the dimerization of two C_{10} hydroxylated fatty acids, and the stereochemistry of this compound was determined to be all (*R*) by base hydrolysis to afford the acids and then acid relactonisation to give the simple C_{10} lactone. The data of this was compared with known compounds.[134] This natural product has moderate activity (MIC 62.5 μg ml^{-1}, SA) and may function as a 'porin' former by localising in the bacterial membrane and causing cell lysis *via* formation of



103

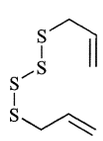
holes in the cell membrane. The final metabolite in this section, aculeatin D (**104**), comes from the ginger family (*Amomum aculeatum*, Zingiberaceae, MIC 8 μg ml^{-1}, SE)[135] and is a dispiro-ether with good cytotoxicity toward KB and L-6 cell lines (IC_{50} = 0.38 and 1 μg ml^{-1} respectively). Furthermore this metabolite was highly antiprotozoal towards *Plasmodium* and *Trypanosoma* species (IC_{50} 0.2–0.49 μg ml^{-1}) and the mechanism of cytotoxicity of this metabolite towards bacterial, mammalian and protozoal cells is unknown.



104

3.10 Sulfur containing products

Oils from the Garlic genus for example garlic itself (*Allium sativum,* Alliaceae) are rich in sulfur containing natural products (*e.g.* allicin) and are known to be strongly antimicrobial.[120] A series of diallyl sulfides, including diallyl tetrasulfide (**105**) have been evaluated[136] using SA and MRSA strains with MIC values of 0.5 and 2.0 μg ml^{-1} respectively. The pure compounds and the parent oils from garlic and Chinese leek (*Allium odorum*) were also active against *Candida* and *Aspergillus* species and are probably produced by *Allium* as latent antimicrobial substances. Ajoene (**106**), a common constituent of *Allium* species, occurs as both the *E* and *Z* isomers and is an inhibitor of platelet aggregation and has potential as a treatment for thrombosis. This metabolite also has anti-staphylococcal activity (bactericidal)[137] with an MIC value of 16 μg ml^{-1} and is additionally antibacterial to species of *Bacillus*, *Mycobacterium* and *Streptomyces*.



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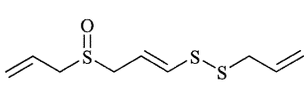
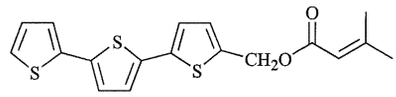
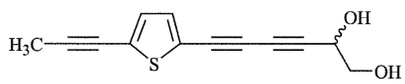


106

When induced by UVA irradiation, terthiophenes have antibiotic activity towards viruses, bacteria, fungi nematodes and eggs and larvae of insects.[138] In a study of nine terthiophenes, typified by **107**, Ciofalo *et al.*,[139] have shown that these compounds are highly active when irradiated (MIC = 0.022 μg ml^{-1} (!), SA, amikacin = 5 μg ml^{-1}) and are inactive at 10 μg ml^{-1} to *Pseudomonas aeruginosa*. This natural product exhibits an astounding level of potency toward *S. aureus* and whilst this activity must be initiated by UV light there may be opportunities to use this class as topical antibacterial agents. A far less



107

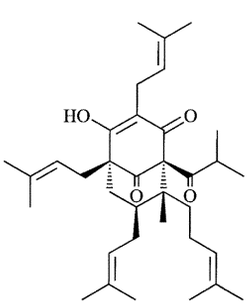


108

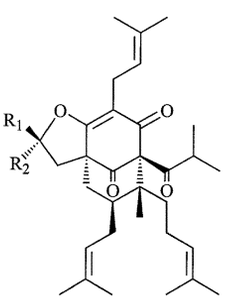
active thiophene-polyyne (**108**) from *Balsamorhiza sagittata* (MIC 25 μg ml^{-1} against MRSA) is also moderately potentiated by exposure to UV light for half an hour.[140]

3.11 Acylphloroglucinols

The acylphloroglucinols are natural products based on an aromatic ring that in many cases has been reduced or has a keto-enol form. The majority of these products are prenylated and/or farnesylated and possess simple acyl groups such as 2-methylpropanoyl which is found in hyperforin (**109**). This metabolite occurs in *Hypericum perforatum* (St John's Wort) and is commonly used as an herbal antidepressant product.[120] Much work has been done on the antibacterial evaluation of hyperforin and *in vitro* activity is exceptional with MIC values ranging from 0.1–1 μg ml^{-1} against penicillin-resistant SA (PRSA) and MRSA strains.[141,142] These results substantiate the use of St John's Wort in several countries as a treatment for superficial burns and wounds that heal poorly.[142] A recent paper describes that exposure of SA to hyperforin leads to a reduced sensitivity to this agent, although the author suggests that resistance cannot be acquired at the doses of which St John's Wort is given for antidepressant effects.[143] Furthermore, the potential use of this agent as an antibiotic is supported by the observation that no resistance occurred at low concentrations of hyperforin and that even in strains with reduced susceptibility, no cross resistance with clinically used antibiotics could be detected.[143] These findings highlight the potential of the acylphloroglucinol class as anti-staphylococcal drug-leads and although this compound is known to be unstable[144] even the degradation products, notably **110** and **111** that occur as a mixture, display moderate activity (50 μg ml^{-1}, SA).[145]

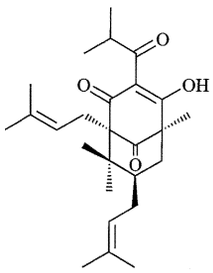


109

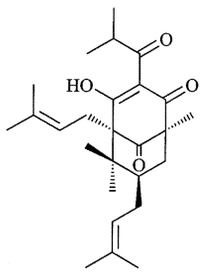


110 R_1 = H, R_2 = OH
111 R_1 = OH, R_2 = H

From the related species, *Hypericum papuanum,* a number of these interesting molecules have been characterised with antibacterial activity.[146,147]Compounds **112** and **113** are tautomeric isomers characterised as a mixture by NMR spectroscopy in the ratio of 4 : 3 respectively (MIC of mixture, 32 μg ml^{-1}, SE). The structure elucidation of these metabolites is not trivial due to the presence of tautomers and in some cases rotameric forms may also be present which can greatly complicate spectra. A

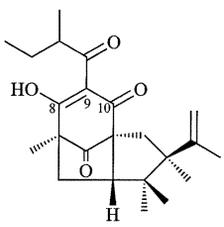


112



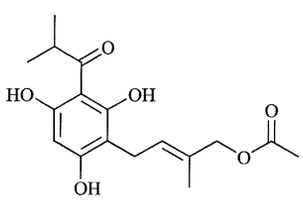
113

further tautomeric mixture from the same plant[147] is seen for **114** (only one form shown) with similar activity towards *S. epidermidis.*



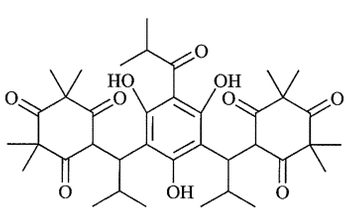
114

Traditional healers in the Free State province of South Africa use *Helichrysum caespititium* as a wound treatment in male circumcision rites and a bioassay-guided study of this species led to the isolation of **115** (MIC, 5 μg ml^{-1}, SA).[148] This compound also inhibited the growth of six fungi, including species of *Aspergillus, Cladosporium* and *Phytophthora,* at low concentration (0.5–5 μg ml^{-1}), indicating an ecological rationale for the presence of this metabolite.



115

Another plant species used in the Mediterranean as an antiseptic is *Myrtus communis,* which produces myrtucommulone A (**116**). This was evaluated using a standard ATCC 25923 SA strain, MRSA possessing the TetK (tetracycline) efflux transporter and further efflux protein producing SA strains, and MIC values ranged from 0.5–2 μg ml^{-1}.[149] The MIC of **116** against SA-1199B which expresses the NorA MDR efflux pump was 1 μg ml^{-1} indicating that myrtucommulone A is not a substrate for this mechanism which would be advantageous in a new class of antibiotic-leads.



116

4 Summary

That no single chemical entity plant-derived anti-staphylococcal agents are used clinically is surprising given the enormous amount of literature on antibacterial extracts and their natural products. It is also puzzling given the use of herbal medicinal products to treat bacterial infections, for example cranberry juice in the management of urinary tract infections.[120,150]

The reasons for this are complex, but probably stem from pharmaceutical companies preferring to pursue microbially derived products, of which there are many first class drug examples which can be readily fermented with few re-supply issues. Additionally, pharmaceutical companies have neglected natural products preferring to utilise combinatorial chemistry libraries as a source of chemical diversity. Unfortunately such libraries lack the true chemical diversity that natural products display (extensive functional group chemistry and chirality) and these libraries are poor for discovery purposes but have potential in lead optimisation. A series of important recent reviews[151] have focused on this deficit and highlight the value of natural products as a screening resource and it is likely that pharmaceutical companies will once again turn their attention to plants, microbes and marine organisms.

Plant sources of antibacterials should not be overlooked as the anti-staphylococcal activities reported in this review are appreciable. Several of the examples such as the acylphloroglucinols and terthiophenes are exceptional and even concerns over re-supply issues could be overcome with access to materials by large-scale cultivation, failing an economically viable synthesis.

What is needed to progress these leads is further profiling against other Gram-positive and Gram-negative bacteria, particularly against resistant, clinically relevant species. In order to interest a pharmaceutical company partner to take on these compounds as development leads, mammalian cell cytotoxicity should be evaluated to see if the margin between bacterial and mammalian cell toxicity can be exploited and ideally small *in vivo* experiments should be conducted to gauge efficacy.

Pressure to find novel antibacterials with new modes of action will drive exploitation of plant sources as antimicrobials. The choice is logical given the ecological rationale that plants produce natural products as a chemical defence against microbes in their environment. Plants produce mammalian cytotoxic compounds *par excellence* and the successes of taxol and taxotere, camptothecin derivatives (topotecan, irinotecan), the vinca alkaloids and the podophyllotoxins to name some of the most successful clinically used anticancer drugs should be justification alone for the exploitation of this chemical pool.

The way is also open for the development of bacterial resistance modifying agents. These could readily mirror the successes seen with RMAs of human tumour resistance, for example inhibitors of p-glycoprotein, one of the major MDR mechanisms. There is also an ecological rationale for the production of natural products that modify microbial resistance. Tegos *et al.*,[125] speculate that plants may have evolved compounds which evade MDR mechanisms and that plant antimicrobials might be developed into broad spectrum antibiotics in combination with inhibitors of MDR. These MDR proteins are, in all probability, commonly found in nature (as efflux pumps to remove foreign toxic substances from the cell) as they are in clinical isolates of resistant pathogens. Further work on organisms which are environmentally relevant to plants is likely to show that these pumps are part of the normal removal of xenobiotics in both bacteria and fungi and it is likely that the compounds that inhibit these processes are also present in the same ecological niche.

5 References

1 S. Gibbons, M. Oluwatuyi and G. W. Kaatz, *J. Antimicrob. Chemother.*, 2003, **51**, 13.

2 T. M. Perl, *Am. J. Med.*, 1999, **106**, 26S–37S.

3 S. S. Rotun, V. McMath, D. J. Schoonmaker, P. S. Maupin, F. C. Tenover, B. C. Hill and D. M. Ackman, *Emerg. Infect. Dis.*, 1999, **5**, 147.

4 M. P. Jevons, *Br. Med. J.*, 1961, **1**, 124.
5 M. P. Jevons, A. W. Coe and M. T. Parker, *Lancet*, 1963, **1**, 904.
6 N. S. Crowcroft and M. Catchpole, *Br. Med. J.*, 2002, **325**, 1390.
7 *'Resistance to Antibiotics' Science and Technology – 7th Report to the House of Lords Committee*, The Stationery Office, London,1998.
8 The National Audit Office, *Report by the Comptroller and Auditor General.* (17th of February), The Stationery Office, London, 2000, pp. 6–121.
9 Public Health Dispatch, *Morbidity Mortality Weekly Rep.*, 2002, **51**, 902.
10 CDC, *Morbidity Mortality Weekly Rep.*, 2002, **51**, 565.
11 S. Tsiodras, H. S. Gold, G. Sakoulas, G. M. Eliopoulos, C. Wennersten, L. Venkataraman, R. C. Moellering and M. J. Ferraro, *Lancet*, 2001, **358**, 207.
12 S. Esposito and S. Noviello, *J. Chemother.*, 1990, **2**, 167.
13 N. J. Marshall and L. J. V. Piddock, *Microbiologia*, 1997, **13**, 285.
14 P. N. Markham, E. Westhaus, K. Klyachko, M. E. Johnson and A. A. Neyfakh, *Antimicrob. Agents Chemother.*, 1999, **43**, 2404.
15 T. S. Yam, J. M. Hamilton-Miller and S. Shah, *J. Antimicrob. Chemother.*, 1998, **42**, 211.
16 J. M. Hamilton-Miller and S. Shah, *FEMS Microbiol. Lett.*, 1999, **176**, 463.
17 J. M. Hamilton-Miller, 1995, Patent Application PCT GB/95/02107.
18 J. M. Hamilton-Miller and S. Shah, *J. Antimicrob. Chemother.*, 2000, **46**, 852.
19 K. Nicolson, G. Evans and P. W. O'Toole, *FEMS Microbiol. Lett.*, 1999, **179**, 233.
20 S. Shiota, M. Shimizu, T. Mizusima, H. Ito, T. Hatano, T. Yoshida and T. Tsuchiya, *FEMS Microbiol. Lett.*, 2000, **185**, 135.
21 M. Shimizu, S. Shiota, T. Mizushima, H. Ito, T. Hatano, T. Yoshida and T. Tsuchiya, *Antimicrob. Agents Chemother.*, 2001, **45**, 3198.
22 S. Gibbons, M. Oluwatuyi, N. C. Veitch and A. I. Gray, *Phytochemistry*, 2003, **62**, 83.
23 S. Gibbons and E. E. Udo, *Phytother. Res.*, 2000, **14**, 139.
24 F. J. Schmitz, A. C. Fluit, M. Luckefahr, B. Engler, B. Hofmann, J. Verhoef, H. P. Heinz, U. Hadding and M. E. Jones, *J. Antimicrob. Chemother.*, 1998, **42**, 807.
25 P. C. Hsieh, S. A. Siegel, B. Rogers, D. Davis and K. Lewis, *Proc. Natl. Acad. Sci. USA*, 1998, **95**, 6602.
26 F. R. Stermitz, P. Lorenz, J. N. Tawara, L. A. Zenewicz and K. Lewis, *Proc. Natl. Acad. Sci. USA*, 2000, **97**, 1433.
27 N. R. Guz and F. R. Stermitz, *J. Nat. Prod.*, 2000, **63**, 1140.
28 F. R. Stermitz, J. Tawara-Matsuda, P. Lorenz, P. Mueller, L. Zenewicz and K. Lewis, *J. Nat. Prod.*, 2000, **63**, 1146.
29 F. R. Stermitz, T. D. Beeson, P. J. Mueller, J. Hsiang and K. Lewis, *Biochem. Syst. Ecol.*, 2001, **29**, 793.
30 F. R. Stermitz, L. N. Scriven, G. Tegos and K. Lewis, *Planta Med.*, 2002, **68**, 1140.
31 C. Morel, F. R. Stermitz, G. Tegos and K. Lewis, *J. Agric. Food Chem.*, 2003, **51**, 5677.
32 F. R. Stermitz, K. K. Cashman, K. M. Halligan, C. Morel, G. P. Tegos and K. Lewis, *Bioorg. Med. Chem. Lett.*, 2003, **13**, 1915.
33 C. T. Price, G. W. Kaatz and J. E. Gustafson, *Int. J. Antimicrob. Agents*, 2002, **20**, 206.
34 I. B. Bassett, D. L. Pannowitz and R. S. Barnetson, *Med. J. Aust.*, 1990, **153**, 455.
35 A. Raman, U. Weir and S. F. Bloomfield, *Lett. Appl. Microbiol.*, 1995, **21**, 242.
36 C. F. Carson and T. V. Riley, *J. Appl. Bacteriol.*, 1995, **78**, 264.
37 S. D. Cox, C. M. Mann and J. L. Markham, *J. Appl. Microbiol.*, 2001, **91**, 492.
38 D. Kalemba, D. Kusewicz and K. Swiader, *Phytother. Res.*, 2002, **16**, 288.
39 R. S. Taylor and G. H. N. Towers, *Phytochemistry*, 1998, **47**, 631.
40 K. H. Lee, T. Ibuka, R. Y. Wu and T. A. Geissman, *Phytochemistry*, 1977, **16**, 1177.
41 K. Kawazoe, Y. Tsubouchi, N. Abdullah, Y. Takaishi, H. Shibata, T. Higuti, H. Hori and M. Ogawa, *J. Nat. Prod.*, 2003, **66**, 538.
42 Y. Sato, H. Oketani, T. Yamada, K. Singyouchi, T. Ohtsubo, M. Kihara, H. Shibata and T. Higuti, *J. Pharm. Pharmacol.*, 1997, **49**, 1042.
43 A. M. Galal, E. A. Abourashed, S. A. Ross, M. A. ElSohly, M. S. Al-Said and F. S. El-Feraly, *J. Nat. Prod.*, 2001, **64**, 399.
44 K. Tamemoto, Y. Takaishi, B. Chen, K. Kawazoe, H. Shibata, T. Higuti, G. Honda, M. Ito, Y. Takeda, O. K. Kodzhimatov and O. Ashurmetov, *Phytochemistry*, 2001, **58**, 763.
45 P. Dolara, B. Corte, C. Ghelardini, A. M. Pugliese, E. Cerbai, S. Menichetti and A. Lo Nostro, *Planta Med.*, 2000, **66**, 356.
46 A. O. Tucker, *Econ. Bot.*, 1986, **40**, 425.
47 D. Y. Shin, H. S. Kim, K. H. Min, S. S. Hyun, S. A. Kim, H. Huh, E. C. Choi, Y. H. Choi, J. Kim, S. H. Choi, W. B. Kim and Y. G. Suh, *Chem. Pharm. Bull. (Tokyo)*, 2000, **48**, 1805.
48 M. I. Aguilar, G. Delgado, M. L. Hernandez and M. L. Villarreal, *Nat. Prod. Lett.*, 2001, **15**, 93.
49 R. Mata, E. Martinez, R. Bye, G. Morales, M. P. Singh, J. E. Janso, W. M. Maiese and B. Timmermann, *J. Nat. Prod.*, 2001, **64**, 911.
50 A. Ulubelen, *Phytochemistry*, 2003, **64**, 395.
51 A. Ulubelen, S. Oksuz, G. Topcu, A. C. Goren and W. Voelter, *J. Nat. Prod.*, 2001, **64**, 549.
52 A. Ulubelen, S. Oksuz, U. Kolak, C. Bozok-Johansson, C. Celik and W. Voelter, *Planta Med.*, 2000, **66**, 458.
53 A. C. Goren, G. Topcu, S. Oksuz, G. Kokdil, W. Voelter and A. Ulubelen, *Nat. Prod. Lett.*, 2002, **16**, 47.
54 A. Ulubelen, S. Oksuz, G. Topcu, A. C. Goren, C. Bozok-Johansson, C. Celik, G. Kokdil and W. Voelter, *Nat. Prod. Lett.*, 2001, **15**, 307.
55 X. Chen, J. Ding, Y. M. Ye and J. S. Zhang, *J. Nat. Prod.*, 2002, **65**, 1016.
56 A. M. El-Lakany, *Pharmazie*, 2003, **58**, 75.
57 O. Batista, M. F. Simoes, A. Duarte, M. L. Valdeira, M. C. de la Torre and B. Rodriguez, *Phytochemistry*, 1995, **38**, 167.
58 J. E. Dellar, M. D. Cole and P. G. Waterman, *Phytochemistry*, 1996, **41**, 735.
59 J. E. Dellar, M. D. Cole and P. G. Waterman, *Biochem. Syst. Ecol.*, 1996, **24**, 83.
60 G. Çitoglu, M. Tanker, B. Sever, J. Englert, R. Anton and N. Altanlar, *Planta Med.*, 1998, **64**, 484.
61 M. Politi, A. Braca, N. De Tommasi, I. Morelli, A. Manunta, L. Battinelli and G. Mazzanti, *Planta Med.*, 2003, **69**, 468.
62 D. Xiao, M. Kuroyanagi, T. Itani, H. Matsuura, M. Udayama, M. Murakami, K. Umehara and N. Kawahara, *Chem. Pharm. Bull. (Tokyo)*, 2001, **49**, 1479.
63 D. Thangadurai, M. B. Viswanathan and N. Ramesh, *Pharmazie*, 2002, **57**, 714.
64 B. Gigante, A. M. Silva, M. J. Marcelo-Curto, S. S. Feio, J. Roseiro and L. V. Reis, *Planta Med.*, 2002, **68**, 680.
65 G. M. Woldemichael, G. Wachter, M. P. Singh, W. M. Maiese and B. N. Timmermann, *J. Nat. Prod.*, 2003, **66**, 242.
66 H. Haraguchi, S. Oike, H. Muroi and I. Kubo, *Planta Med.*, 1996, **62**, 122.
67 G. B. Evans and R. H. Furneaux, *Bioorg. Med. Chem.*, 2000, **8**, 1653.
68 H. Muroi and I. Kubo, *Biosci. Biotech. Biochem.*, 1994, **58**, 1925.
69 I. Kubo, H. Muroi and M. Himejima, *J. Nat. Prod.*, 1992, **55**, 1436.
70 J. R. Zgoda-Pols, A. J. Freyer, L. B. Killmer and J. R. Porter, *Fitoterapia*, 2002, **73**, 434.
71 A. Zamilpa, J. Tortoriello, V. Navarro, G. Delgado and L. Alvarez, *Planta Med.*, 2002, **68**, 281.
72 S. Erazo, M. Zaldivar, C. Delporte, N. Backhouse, P. Tapia, E. Belmonte, F. Delle Monarche and R. Negrete, *Planta Med.*, 2002, **68**, 361.
73 B. M. Tincusi, I. A. Jimenez, I. L. Bazzocchi, L. M. Moujir, Z. A. Mamani, J. P. Barroso, A. G. Ravelo and B. V. Hernandez, *Planta Med.*, 2002, **68**, 808.
74 V. Roussis, M. Tsoukatou, I. B. Chinou and A. Ortiz, *Planta Med.*, 1998, **64**, 675.
75 A. Ankli, J. Heilmann, M. Heinrich and O. Sticher, *Phytochemistry*, 2000, **54**, 531.
76 G. M. Woldemichael, M. P. Singh, W. M. Maiese and B. N. Timmermann, *Z. Naturforsch., C: Biosci.*, 2003, **58**, 70.
77 A. Braca, I. Morelli, J. Mendez, L. Battinelli, L. Braghiroli and G. Mazzanti, *Planta Med.*, 2000, **66**, 768.
78 K. Y. Orabi, S. I. Al-Qasoumi, M. M. El-Olemy, J. S. Mossa and I. Muhammad, *Phytochemistry*, 2001, **58**, 475.
79 D. R. Katerere, A. I. Gray, R. J. Nash and R. D. Waigh, *Phytochemistry*, 2003, **63**, 81.
80 I. Muhammad, K. A. El Sayed, J. S. Mossa, M. S. Al-Said, F. S. El-Feraly, A. M. Clark, C. D. Hufford, S. Oh and A. M. S. Mayer, *J. Nat. Prod.*, 2000, **63**, 605.
81 M. T. Gutierrez-Lugo, M. P. Singh, W. M. Maiese and B. N. Timmermann, *J. Nat. Prod.*, 2002, **65**, 872.
82 M. Chatterji, S. Unniraman, S. Mahadevan and V. Nagaraja, *J. Antimicrob. Chemother.*, 2001, **48**, 479.
83 Y. Tada, Y. Shikishima, Y. Takaishi, H. Shibata, T. Higuti, G. Honda, M. Ito, Y. Takeda, O. K. Kodzhimatov, O. Ashurmetov and Y. Ohmoto, *Phytochemistry*, 2002, **59**, 649.
84 W. N. Setzer, M. C. Setzer, R. B. Bates, P. Nakkiew, B. R. Jackes, L. Chen, M. B. McFerrin and E. J. Meehan, *Planta Med.*, 1999, **65**, 747.
85 P. M. Pauletti, A. R. Araujo, M. C. Young, A. M. Giesbrecht and V. D. Bolzani, *Phytochemistry*, 2000, **55**, 597.
86 K. Kawazoe, A. Yutani, K. Tamemoto, S. Yuasa, H. Shibata, T. Higuti and Y. Takaishi, *J. Nat. Prod.*, 2001, **64**, 588.
87 E. Kamory, G. M. Keseru and B. Papp, *Planta Med.*, 1995, **61**, 387.

88 J. R. Zgoda-Pols, A. J. Freyer, L. B. Killmer and J. R. Porter, *J. Nat Prod.*, 2002, **65**, 1554.
89 I. Muhammad, X. C. Li, D. C. Dunbar, M. A. ElSohly and I. A. Khan, *J. Nat. Prod.*, 2001, **64**, 1322.
90 I. Muhammad, X. C. Li, M. R. Jacob, B. L. Tekwani, D. C. Dunbar and D. Ferreira, *J. Nat. Prod.*, 2003, **66**, 804.
91 J. Orjala, P. Mian, T. Rali and O. Sticher, *J. Nat. Prod.*, 1998, **61**, 939.
92 C. C. Shen, W. J. Syu, S. Y. Li, C. H. Lin, G. H. Lee and C. M. Sun, *J. Nat. Prod.*, 2002, **65**, 1857.
93 Y. Morita, E. Matsumura, H. Tsujibo, M. Yasuda, T. Okabe, Y. Sakagami, Y. Kumeda, N. Ishida and Y. Inamor, *Biol. Pharm. Bull.*, 2002, **25**, 981.
94 Y. Sato, H. Oketani, K. Singyouchi, T. Ohtsubo, M. Kihara, H. Shibata and T. Higuti, *Biol. Pharm. Bull.*, 1997, **20**, 401.
95 K. Kono, I. Tatara, S. Takeda, K. Arakawa and Y. Hara, *Kansenshogaku Zasshi*, 1994, **68**, 1518.
96 R. Tsukiyama, H. Katsura, N. Tokuriki and M. Kobayashi, *Antimicrob. Agents Chemother.*, 2002, **46**, 1226.
97 T. Fukai, A. Marumo, K. Kaitou, T. Kanda, S. Terada and T. Nomura, *Fitoterapia*, 2002, **73**, 536.
98 A. S. Joshi, X. C. Li, A. C. Nimrod, H. N. ElSohly, L. A. Walker and A. M. Clark, *Planta Med.*, 2001, **67**, 186.
99 Y. Sato, S. Suzaki, T. Nishikawa, M. Kihara, H. Shibata and T. Higuti, *J. Ethnopharmacol.*, 2000, **72**, 483.
100 M. M. Rahman and A. I. Gray, *Phytochemistry*, 2002, **59**, 73.
101 N. P. Nanayakkara, C. L. Burandt Jr. and M. R. Jacob, *Planta Med.*, 2002, **68**, 519.
102 S. G. Dastidar, S. K. Mahapatra, K. Ganguly, A. N. Chakrabarty, Y. Shirataki and N. Motohashi, *In Vivo*, 2001, **15**, 519.
103 Y. Sakagami, M. Mimura, K. Kajimura, H. Yokoyama, M. Linuma, T. Tanaka and M. Ohyama, *Lett. Appl. Microbiol.*, 1998, **27**, 98.
104 N. Didry, L. Dubreuil and M. Pinkas, *Phytother. Res.*, 1993, **7**, 242.
105 M. Kuroyanagi, T. Arakawa, Y. Hirayama and T. Hayashi, *J. Nat. Prod.*, 1999, **62**, 1595.
106 Y. Deng, J. P. Lee, M. Tianasoa-Ramamonjy, J. K. Snyder, S. A. Des Etages, D. Kanada, M. P. Snyder and C. J. Turner, *J. Nat. Prod.*, 2000, **63**, 1082.
107 M. Sato, H. Tsuchiya, T. Miyazaki, M. Ohyama, T. Tanaka and M. Iinuma, *Lett. Appl. Microbiol.*, 1995, **21**, 219.
108 A. E. Nkengfack, J. C. Vardamides, Z. T. Fomum and M. Meyer, *Phytochemistry*, 1995, **40**, 1803.
109 A. K. Waffo, G. A. Azebaze, A. E. Nkengfack, Z. T. Fomum, M. Meyer, B. Bodo and F. R. van Heerden, *Phytochemistry*, 2000, **53**, 981.
110 H. Tanaka, T. Oh-Uchi, H. Etoh, M. Sako, F. Asai, T. Fukai, M. Sato, J. Murata and Y. Tateishi, *Phytochemistry*, 2003, **64**, 753.
111 H. Tanaka, M. Sato, S. Fujiwara, M. Hirata, H. Etoh and H. Takeuchi, *Lett. Appl. Microbiol.*, 2002, **35**, 494.
112 J. B. Harborne, *Introduction to Ecological Biochemistry*, Academic Press Publishers, London, 1994, 4th Edition.
113 R. R. Majinda, M. Motswaledi, R. D. Waigh and P. G. Waterman, *Planta Med.*, 1997, **63**, 268.
114 S. Tang, P. Bremner, A. Kortenkamp, C. Schlage, A. I. Gray, S. Gibbons and M. Heinrich, *Planta Med.*, 2003, **69**, 247.
115 A. Chakraborty, C. Saha, G. Podder, B. K. Chowdhury and P. Bhattacharyya, *Phytochemistry*, 1995, **38**, 787.
116 K. Nakahara, G. Trakoontivakorn, N. S. Alzoreky, H. Ono, M. Onishi-Kameyama and M. Yoshida, *J. Agric. Food Chem.*, 2002, **50**, 4796.
117 K. Cimanga, T. De Bruyne, A. Lasure, B. Van Poel, L. Pieters, M. Claeys, D. V. Berghe, K. Kambu, L. Tona and A. J. Vlietinck, *Planta Med.*, 1996, **62**, 22.
118 H. A. Saad, S. H. El-Sharkawy and W. T. Shier, *Planta Med.*, 1995, **61**, 313.
119 A. L. Okunade, A, M. Clark, C. D. Hufford and B. O. Oguntimein, *Planta Med.*, 1999, **65**, 447.
120 J. E. Barnes, L. A. Anderson and J. D. Phillipson, *Herbal Medicine: A Guide for Health Care Professionals*, Pharmaceutical Press, London, 2002, 2nd edition.
121 *Trease and Evans Pharmacognosy*, ed. W. C. Evans, W. B. Saunders Publishers, London, 2002, 15th Edition, p. 363.
122 F. Scazzocchio, M. F. Cometa, L. Tomassini and M. Palmery, *Planta Med.*, 2001, **67**, 561.
123 S. Gibbons, J. Leimkugel, M. Oluwatuyi and M. Heinrich, *Phytother. Res.*, 2003, **17**, 274.
124 Z. Zhang, H. N. ElSohly, M. R. Jacob, D. S. Pasco, L. A. Walker and A. M. Clark, *J. Nat. Prod.*, 2002, **65**, 856.
125 G. Tegos, F. R. Stermitz, O. Lomovskaya and K. Lewis, *Antimicrob. Agents Chemother.*, 2002, **46**, 3133.
126 X. C. Li, D. C. Dunbar, H. N. ElSohly, M. R. Jacob, A. C. Nimrod, L. A. Walker and A. M. Clark, *J. Nat. Prod.*, 2001, **64**, 1153.
127 H. R. Dharmaratne, W. M. Wijesinghe and V. J. Thevanasem, *J. Ethnopharmacol.*, 1999, **66**, 339.
128 M. Iinuma, H. Tosa, T. Tanaka, F. Asai, Y. Kobayashi, R. Shimano and K. Miyauchi, *J. Pharm. Pharmacol.*, 1996, **48**, 861.
129 A. E. Nkengfack, P. Mkounga, M. Meyer, Z. T. Fomum and B. Bodo, *Phytochemistry*, 2002, **61**, 181.
130 S. Nakamura and A. Hatanaka, *J. Agric. Food. Chem.*, 2002, **50**, 7639.
131 J. R. Zgoda, A. J. Freyer, L. B. Killmer and J. R. Porter, *J. Nat. Prod.*, 2001, **64**, 1348.
132 H. Matsuura, G. Saxena, S. W. Farmer, R. E. Hancock and G. H. N. Towers, *Planta Med.*, 1996, **62**, 256.
133 D. Lechner, M. Stavri, M. Oluwatuyi, R. Pereda-Miranda and S. Gibbons, *Phytochemistry*, 2004, **64**, 331.
134 P. Magiatis, D. Spanakis, S. Mitaku, E. Tsitsa, A. Mentis and C. Harvala, *J. Nat. Prod.*, 2001, **64**, 1093.
135 J. Heilmann, R. Brun, S. Mayr, T. Rali and O. Sticher, *Phytochemistry*, 2001, **57**, 1281.
136 S. M. Tsao and M. C. Yin, *J. Med. Microbiol.*, 2001, **50**, 646.
137 R. Naganawa, N. Iwata, K. Ishikawa, H. Fukuda, T. Fujino and A. Suzuki, *Appl. Environ. Microbiol.*, 1996, **62**, 4238.
138 J. Kagan, *Prog. Chem. Org. Nat. Prod.*, 1991, **56**, 87.
139 M. Ciofalo, S. Petruso and D. Schillaci, *Planta Med.*, 1996, **62**, 374.
140 H. Matsuura, G. Saxena, S. W. Farmer, R. E. Hancock and G. H. N. Towers, *Planta Med.*, 1996, **62**, 65.
141 C. M. Schempp, K. Pelz, A. Wittmer, E. Schopf and J. C. Simon, *Lancet*, 1999, **353**, 2129.
142 J. Reichling, A. Weseler and R. Saller, *Pharmacopsychiatry*, 2001, **34**(Suppl 1), S116.
143 A. T. Hübner, *Phytomedicine*, 2003, **10**, 206.
144 S. Trifunovic, V. Vajs, S. Macura, N. Juranic, Z. Djarmati, R. Jankov and S. Milosavljevic, *Phytochemistry*, 1998, **49**, 1305.
145 V. Vajs, S. Vugdelija, S. Trifunovic, I. Karadzic, N. Juranic, S. Macura and S. Milosavljevic, *Fitoterapia*, 2003, **74**, 439.
146 K. Winkelmann, J. Heilmann, O. Zerbe, T. Rali and O. Sticher, *J. Nat. Prod.*, 2001, **64**, 701.
147 K. Winkelmann, J. Heilmann, O. Zerbe, T. Rali and O. Sticher, *J. Nat. Prod.*, 2000, **63**, 104.
148 A. D. Mathekga, J. J. M. Meyer, M. M. Horn and S. E. Drewes, *Phytochemistry*, 2000, **53**, 93.
149 G. Appendino, F. Bianchi, A. Minassi, O. Sterner, M. Ballero and S. Gibbons, *J. Nat. Prod.*, 2002, **65**, 334.
150 A. Stapleton, *Infect. Dis. Clin. North Am.*, 2003, **17**, 457.
151 A. M. Rouhi, *Chem. Eng. News*, 2003, **81**, pp. 77, 93 and 104.