Naphthopyranones - isolation, bioactivity, biosynthesis and synthesis

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Naphthopyranones – isolation, bioactivity, biosynthesis and synthesis

Christopher D. Donner*

The 1H-naphtho[2,3-c]pyran-1-one (naphthopyranone) moiety forms the structural framework of a group of secondary metabolites that have been isolated from a range of organisms including fungi, bacteria, lichen and plants. This review documents the known naturally occurring naphthopyranones – their isolation, biosynthesis and biological activity. A survey of methods reported for the synthesis of naphthopyranone natural products is presented.

1. Introduction

Natural products that include a naphthopyranone (1H-naphtho[2,3-c]pyran-1-one) core structure A are widely dispersed in Nature, having been isolated from a variety of sources including fungi, bacteria, lichen and plants. The bioactivity of this group, often the directing force for isolation of these compounds, encompasses antibiotic, cytotoxic, antioxidant, immunoregulatory, antimalarial and antifungal activities amongst the diverse range of identified bioactivities. Given that natural products and natural product-inspired compounds still represent a significant proportion of currently available pharmaceuticals, investigation of this structure class for potential lead-compounds in the development of pharmaceuticals or for use as biochemical probes warrants their ongoing exploration.

Figure 1: Typical core structural motifs A-C of naphthopyranone natural products (the numbering depicted in A is commonly adopted and is used for consistency throughout this discussion).
The structural framework A defining this group is depicted in Figure 1 and can be seen to consist of a linear tricyclic system incorporating naphthalene and fused δ-lactone (α-pyrone) rings. The structure A is an extension of the bicyclic isocoumarin (1H-benzopyran-1-one) skeleton, the later of which often co-occur with naphthopyranone natural products. It is worth noting that natural products based on the isomeric naphthopyran-4-one (γ-pyrone) structure also regularly occur in nature, a detailed discussion of which is available. Oxidation in A is typically present at the C-7, C-9 and C-10 positions as a consequence of their polyketide biosynthetic origin. More often they exist in the 3,4-dihydro form B, with the presence of an alkyl group at C-3 imparting asymmetry to the structures. Oxidation of the naphthalene to a naphthoquinone, as in the 6,9-quinone C, is a regularly encountered modification of the parent structure. Furthermore, both symmetrical and unsymmetrical dimerization of naphthopyranones at C-6, C-8 and/or through ether linkages provides further structural and stereochemical diversity within this family. A small number of ring-fused naphthopyranone derivatives, as exemplified by structures D-G (Figure 2), are known to occur naturally and have been included in the current discussion.

![Figure 2: Ring-fused variations D-G of the naphthopyranone core structure.](image)

Early investigations of naphthopyranones often focussed on the quinone-containing members of the family, likely as a consequence of these pigments being responsible for the colouration displayed by the organism (e.g. fungi) from which they were sourced. Later, with bioactivity-guided isolation of natural products being more prevalent, newer and more diverse members of the naphthopyranone family of natural products have been identified. Not only do members of the family have potential in the area of medicinal chemistry, additionally, their widespread occurrence across a variety species has made them very useful biomarkers in the area of taxonomic identification.

This review provides an overview of known naturally occurring naphthopyranones, exemplifying the structural variety based on the core structures A-G, along with known bioactivity of members of this group. A discussion of the reported syntheses of members of this group is given with particular emphasis on the use of synthesis for the confirmation of structure, resolution of structural uncertainties such as stereochemistry and for the investigation of biosynthesis. For the purposes of the following discussion members of the naphthopyranone family of natural products have been arbitrarily classified as belonging to one of five sub-groups based on certain structural features that they possess. These are:

1. Toralactone-based naphthopyranones ('A-type', Fig. 1);
2. Naphthopyranones asymmetric at C-3 ('B-type', Fig. 1);
3. Oxidized (quinonoid) naphthopyranones ('C-type', Fig. 1);
4. Naphthopyranones coupled to a non-naphthopyranone moiety; and
5. Naphthopyranones having extended ring systems ('D-G-type', Fig. 2).

In addition, naphthopyranones isolated as a result of engineered polyketide synthesis are included as a separate group. Some structures, in particular the heterodimeric and quinonoid species, can be considered to belong to more than one sub-group and, as such, the separation into these groups is merely to assist in the discussion and ease of comparison of these natural products.

2. Isolation and bioactivity

2.1 Toralactone-based naphthopyranones

Toralactone I was first isolated in 1973 as a constituent of the seeds of Cassia tora L.6 then later isolated from C. obtusifolia L.7 and is also found as a metabolite in a cultivated mycobiont of the lichen Pyrenula sp.8 Toralactone I represents the quintessential structure of the naphthopyranone group of compounds and itself displays antimicrobial activity. A series of glycosides of toralactone I are known, including the 9-O-glucoside 2 that has been obtained from Eriocaulon buergerianum,9 E. ligulatum10 and Puepalanthus chiquitensis11 along with numerous other naphthopyranones, flavonoids and xanthones. Eriocaulon buergerianum ("Gujingeao") is used in traditional Chinese medicine for its ophthalmic, anti-inflammatory and antimicrobial effects. Toralactone glucoside 2 was shown to have α-glucosidase inhibitory activity (IC50 106.7 µM).12

[[STRUCTURES 1-16 TO BE INSERTED HERE]]

From the seeds of Cassia obtusifolia L. have been isolated the gentiobiocide cassiaside C313 and cassiaside C4.14 Cassiaside C2 4 inhibits histamine release from rat peritoneal exudate mast cells induced by an antigen-antibody reaction. Cassiaside C 3 and cassiaside C2 4 also occur in C. tora and were shown to possess negligible antibacterial effects against Pseudomonas aeruginosa and four strains of MRSA.15 However, the aglycone toralactone 1 showed significant activity (MIC of 64 µg/mL) against the MRSA strains tested. Accompanying 3 and 4 in Cassia tora is a further member of this series of toralactone glycosides, triglucoside 5, isolated as part of a bioassay-guided study searching for plant compounds
with estrogen-like activity.\textsuperscript{16} In comparing a series of natural and synthetic phenolic compounds the estrogen-like activity of cassiaside C \textsuperscript{3} was modest, whereas the aglycone toralactone \textsuperscript{1} was shown to exhibit significant estrogenic activity. Additionally, synthetically-derived nor-toralactone (obtained by cleaving the C-7 methyl ether of toralactone \textsuperscript{1}) showed further enhancement of estrogenic activity in an estrogen-dependent MCF-7 cell-proliferation assay and also anti-estrogenic activity in a concentration dependent manner.

From the roots of \textit{Cassia torosa} CAV., but not in the seeds and seedlings of this plant, were isolated 8-methyltoralactone \textsuperscript{6} and the corresponding 10-O-methyl ether \textsuperscript{7}, along with several phytosterols and anthraquinone pigments.\textsuperscript{17} The location of the methyl group at C-8 was established from a series of NOE experiments and derivatization. The cassia plant has a long history as a traditional medicine in Asian countries such as Japan, China and Korea where it has been used as a diuretic, laxative, antiasthenic and for the treatment of conditions such as hepatitis and eye disease. Whilst toralactone \textsuperscript{1} and its various glycosides contribute to the activity of cassia seeds and extracts, other components including anthraquiones and hydroanthracenes within the plant may also have important roles.\textsuperscript{18}

Isotoralactone \textsuperscript{8} was isolated from the seeds of \textit{Cassia obtusifolia} L. and the structure established from spectral analysis and derivatization.\textsuperscript{19} Thus, upon acetylation of \textsuperscript{8} a diacetate was formed and shown to be identical with the diacetate of toralactone \textsuperscript{1}, indicating migration of the double-bond takes place under these conditions. Isomerization of isotoralactone \textsuperscript{8} to toralactone \textsuperscript{1} could be effected by treatment with acid. Both isotoralactone \textsuperscript{8} and toralactone \textsuperscript{1} display antimicrobial activity against \textit{Staphylococcus aureus} (MIC 2-3 \textmu g/mL) and \textit{Escherichia coli} (MIC 6-12 \textmu g/mL)\textsuperscript{7} and show only moderate inhibitory activity towards cAMP phosphodiesterase.\textsuperscript{20}

The chemical constituents of the Eriocaulaceae plant family have been shown to be highly informative in taxonomic studies. In particular, the occurrence of naphthopyranone and flavonoid derivatives in \textit{Paepalanthus} species has allowed these natural products to be used effectively as taxonomic markers for identification of genera in the Eriocaulaceae family.\textsuperscript{21} Paepalantine \textsuperscript{9} is the main constituent in the capitula of \textit{Paepalanthus bromelioides}, an ornamental plant native to Brazil, and shows antimicrobial activity against various bacteria including \textit{Bacillus cereus} (MIC 7.5 \textmu g/mL), \textit{Staphylococcus aureus} (MIC 7.5 \textmu g/mL) and \textit{S. epidermidis} (MIC 15 \textmu g/mL).\textsuperscript{22} Paepalantine \textsuperscript{9} has also been isolated from \textit{Paepalanthus vellozioides} and shown to be mutagenic in CHO and McCoy cell assays,\textsuperscript{23} cytotoxic in both \textit{in vitro} and \textit{in vivo} mammalian systems\textsuperscript{24} and protects mitochondria exposed to oxidative stress conditions by scavenging of superoxide radicals.\textsuperscript{25} The capitula of \textit{Paepalanthus bromelioides} also contains the glycosides \textsuperscript{10} and \textsuperscript{11},\textsuperscript{26} whilst the aerial parts of \textit{P. microphyllus} contains \textsuperscript{10} and \textsuperscript{12-14}.\textsuperscript{27} A number of the glycosides of paepalantine have also been assessed for cytotoxic\textsuperscript{28} and mutagenic\textsuperscript{29} activity.

The flowers of \textit{Paepalanthus geniculatus} contain naphthopyranone glycosides \textsuperscript{15} and \textsuperscript{16}, the 7-de-O-methyl analogues of \textsuperscript{10} and \textsuperscript{12}, along with paepalantine glycosides \textsuperscript{11} and \textsuperscript{12}.\textsuperscript{30} The additional free hydroxy groups in \textsuperscript{15} and \textsuperscript{16} enhance the radical-scavenging capacity in comparison to \textsuperscript{10} and \textsuperscript{12}, as measured using the trolox equivalent antioxidant capacity (TEAC) assay.

The C-8/C-8' coupled dehydro dimer \textsuperscript{17} of paepalantine has been isolated from \textit{P. bromelioides} and has an \( IC_{50} \) of 55.9 \mu M on colorimetric assay for toxicity.\textsuperscript{31} The paepalantine dimer \textsuperscript{17} inhibits human kallikrein 5 and 7 serine proteases, the over-expression of which occurs in endocrine-related malignancies, with \( K_i \) values of 24.4 \mu M and 12.2 \mu M, respectively.\textsuperscript{32}

\textbf{2.2 Naphthopyranones asymmetric at C-3}

Eriocauline \textsuperscript{18}, a C-10/C-10' ether-linked dimer of toralactone \textsuperscript{1}, has been isolated from the dichloromethane extracts from capitulae of \textit{Eriocaulon ligulatum},\textsuperscript{33} an ornamental flower exported from Brazil. Whilst not possessing an asymmetric centre, circular dichroism (CD) and specific rotation data suggest that eriocauline \textsuperscript{18} displays atropisomerism. The dichloromethane extract from \textit{E. ligulatum}, in which there is a prevalence of eriocauline \textsuperscript{18}, showed mutagenic activity (Ames test) in one of the four strains of \textit{Salmonella typhimurium} tested.

Naphthopyranones, particularly toralactone \textsuperscript{1} and paepalantine \textsuperscript{9} and their various glycosides, along with other co-metabolites such as flavonoids and xanthones have been used as reliable markers for the identification of \textit{Eriocaulon} species using techniques such as high-performance liquid chromatography (HPLC) and mass spectrometry (MS).\textsuperscript{34} The rapid throughput and ability to identify known compounds using the combined techniques of HPLC, HRMS, MS/MS and UV-absorption also serves as a valuable tool for dereplication during the search for new bioactive compounds from fungal sources.\textsuperscript{35,36}
Cassialactone 19 was isolated, along with isotalactone 8, from the seeds of Cassia obtusifolia.\textsuperscript{19} The originally proposed structure for cassialactone was revised to 19 on the basis of X-ray analysis of the triacetate derivative.\textsuperscript{37} The (S)-configuration at C-3 in 19 was established by analysis of the CD spectrum of a \( p \)-methoxybenzoyl derivative. Cassialactone 19, and a related dimeric species that incorporates C-8-methyl cassialactone, appear to be the only naturally occurring naphthopyranones that are disubstituted at C-3.

\( \left( R \right) \)-Semivioxanthin 20 was first identified as a metabolite of Penicillium citreo-viride\textsuperscript{28} and the stereochemistry deduced by comparison of the CD spectrum to that of the isocoumarin (R)-mellein. \( \left( R \right) \)-Semivioxanthin 20 has also been found in Aspergillus ochraceus LCI11-102 associated with the coral Dichotella gemmacea,\textsuperscript{39} the endophytic fungus Aspergillus sp. HS-05 isolated from leaves of Huperzia serrata\textsuperscript{40} and also in capitulae of the plant Eriocaulon buergerianum.\textsuperscript{34} Notably, the enantiomeric species (S)-semivioxanthin 24 is also known to occur naturally having been isolated recently from mycelium of Cryptosporiopsis abietina,\textsuperscript{41} the fungus responsible for stem canker of the Hinoki cypress in Japan. (S)-Semivioxanthin 24 showed abscisic activity against Hinoki cypress leaves and inhibited spore germination of the fungus Cladosporium herbarum. Semivioxanthin (of unspecified stereochemistry) isolated from an unidentified marine-derived fungus showed immunostimulatory effects resulting from promotion of tumor necrosis factor (TNF)-\( \alpha \) production through nuclear factor (NF)-\( \kappa B \) and mitogen-activated protein kinase (MAPK) signalling pathways.\textsuperscript{42}

Further structural modification of semivioxanthin, such as glycosylation or biaryl coupling, has commonly taken place prior to isolation. For example, the glycosides 21-23 of \( \left( R \right) \)-semivioxanthin occur in leaves of the Brazilian plants Paepalanthus vellozioides and P. latipes\textsuperscript{43} and also in the Chinese plant Eriocaulon buergerianum.\textsuperscript{9} The \( \left( R \right) \)-stereochemistry at C-3 in 22 and 23 was established by comparison of the CD spectra of the corresponding aglycones with \( \left( R \right) \)-semivioxanthin 20.

Vioxanthin 26 was first isolated in 1966 from Trichophyton violaceum,\textsuperscript{45} and has since been isolated from Penicillium citreo-viride,\textsuperscript{38} P. aurantiogriseum obtained from cereal samples,\textsuperscript{46} Aspergillus ochraceus\textsuperscript{47} and the thermophilic fungus Malbranchea pulchella var. sulfurea isolated from compost.\textsuperscript{48} Vioxanthin 26 isolated from the later source was labelled \(' T '\)-26Vx and shown to be highly active against Gram-positive bacteria (MIC range of 0.045-1.56 \( \mu \)g/mL) and strictly anaerobic Gram-negative bacteria. Initially the \( \left( P,R,R \right) \)-configuration\textsuperscript{49} of vioxanthin 26 obtained from Penicillium citreo-viride was proposed by Zeeck and co-workers based on interpretation of the CD spectrum and co-occurrence of \( \left( R \right) \)-semivioxanthin 20.\textsuperscript{48} The CD spectrum of 26 shows a strong positive Cotton effect to longer wavelength and a strong negative one to shorter wavelength that corresponds to ‘positive exciton chirality’,\textsuperscript{50} which in the case of 26 corresponds to a \( P \)-configuration at the stereogenic axis according to the Prelog-Helmchen rules. This conclusion has been rigorously confirmed by a combination of total synthesis and feeding experiments using \( ^{13} \)C-labelled 7-de-O-methylsemivioxanthin.\textsuperscript{51} Application of the exciton chirality method, or comparison of CD spectra to that of vioxanthin 26, has routinely been used to deduce the axial chirality of dimeric naphthopyranones.

Pigmentosin A 27, a 6,6’-binaphthopyranone, was obtained from the lichen Hypotrachyna immaculata collected in Australia and shown to be the regiosomer of vioxanthin 26.\textsuperscript{52}

In this regard, HMBC and HMQC correlations and a significant upfield shift of the H-5 proton signal in the \( ^{1} \)H NMR spectrum of 27 due to shielding by the adjacent aromatic ring established the site of connectivity. The recent stereoselective synthesis of pigmentosin A 27 has established the absolute stereochemistry depicted.\textsuperscript{53} The synthetic material was shown to inhibit the growth of Bacillus subtilis with an MIC of 20 \( \mu \)M. The regiosomers pigmentosin A 27 and vioxanthin 26 have been isolated as co-metabolites in the lichen Hypotrachyna toiana found in Malaysia.\textsuperscript{54}

Another pair of regioisomeric binaphthopyranones found as co-metabolites are aschernaphthopyrones A 28 and B 29.\textsuperscript{55}

Isolated from the scale insect pathogenic fungus Aschersonia paraphysata, 28 and 29 are the C-7 de-O-methyl analogues of pigmentosin A 27 and vioxanthin 26, respectively. Whilst the axial stereochemistry of aschernaphthopyrones A 28 and B 29 could be determined by interpretation of their CD spectra, the absolute stereochemistry at C-3/C-3’ in 28 and 29 is yet to be established. Aschernaphthopyrone A 28 shows moderate...
antimalarial activity against *Plasmodium falciparum* K1 with an IC\textsubscript{50} of 7.3 µM in addition to cytotoxicity against NCI-H187 cells (human small-cell lung cancer) and KB cells (oral human epidermoid carcinoma) with IC\textsubscript{50} values of 4.5 µM and 17 µM, respectively. Aschernaphthopyrone B 29 showed much weaker activity in similar assays.

The antitumor antibiotics PF1158A 30, PF1158B 31 and PF1158C 32, produced in the culture fluids of *Geosmithia argillacea* PF1158, are inhibitors of P388 mouse leukemia cells.\textsuperscript{56} The antibiotic compounds 30-32 are novel trimers composed of three semivioxanthin moieties.

[[STRUCTURES 30-32 TO BE INSERTED HERE]]

The ether bridged naphthopyranone dimer planifolin 33 is found in the capitula of *Paepalanthus planifolius*.\textsuperscript{57} The two components of planifolin 33 correspond to semivioxanthin 20 and paepalantine 9. The stereochemistry at C-3 in the semivioxanthin moiety of planifolin 33 was assumed to be (R) due to the presence of various glycosides of (R)-semivioxanthin 20 in several other *Paepalanthus* species. Planifolin 33 shows mutagenic activity, as measured using the Ames test, and cytotoxicity to cells of the McCoy mouse fibroblast cell line.\textsuperscript{58} The cytotoxicity index (Cl\textsubscript{50}) for planifolin of 12.8 µg/mL compares favourably to cisplatin (Cl\textsubscript{50} 41.9 µg/mL).

The fungus *Paecilomyces variotii*, isolated from the larvae of the mountain pine beetle *Dendroctonus ponderosae*, produces the metabolites semiviriditoxin 36 and semiviriditoxic acid 37.\textsuperscript{61} The structures of which were established by using chemical and spectroscopic methods. The (S)-stereochemistry was deduced in both cases by comparison of their CD spectra with that of (R)-semivioxanthin 20.

Viriditoxin 38, the C-6/C-6'- dehydro dimer of semiviriditoxin 36, occurs in several fungi including *Aspergillus viridinutans*,\textsuperscript{62} *A. brevipes*,\textsuperscript{63} *A. fumigatus*\textsuperscript{64} and *Spicaria divaricata*.\textsuperscript{65} It was erroneously assigned a C-8/C-8' coupled structure that was subsequently revised on the basis of difference NOE experiments involving viriditoxin tetramethyl ether.\textsuperscript{66} These showed correlation between H-8 and both the C-7 and C-9 methoxy groups. The chirality of the C-6/C-6' axis in 38 was established from the CD spectrum. Recently, the absolute configuration at the stereogenic centres C-3 and C-3' in 38 has been determined by total synthesis.\textsuperscript{67} Viriditoxin 38 has an LD\textsubscript{50} of 2.8 mg/kg in mice,\textsuperscript{62} and is reported to block the polymerization of FtsZ, a tubulin-like GTPase that has an essential role in bacterial cell division, with an IC\textsubscript{50} of 8.2 µg/mL.\textsuperscript{68} However, a later study has shown that the conditions required to observe inhibition of FtsZ polymerization by
viriditoxin 38 are very specific and that the toxicity of this compound may preclude its further development in drug discovery. Viriditoxin 38 shows activity against a range of bacterial pathogens including methicillin-resistant and methicillin-sensitive strains of Staphylococcus aureus (MIC 4-8 µg/mL) and vancomycin-resistant and vancomycin-sensitive Enterococcus faecalis and Enterococcus faecium (MIC 2-16 µg/mL).  

Asteromine 39 was found in a strain of the fungus Mycosphaerella asteroma. It is phytotoxic to Cucumis sativus (cucumber), weakly antibacterial and antifungal and is lethal to Artemia salina shrimp at 0.1 mM. The structure and axial chirality of asteromine 39 was established by spectroscopic analysis of the dimethyl ester, however, the stereochemistry at C-3 and C-3' is not known.

SC-28763 40 and SC-30532 41 were isolated from Spicaria divaricata, along with the related metabolite SC-28762 that is structurally identical to viriditoxin 38. Originally proposed as having a C-8/C-8' biaryl axis, structural revision to the depicted C-6/C-6' coupled systems 40 and 41 has been proposed in line with the revision of the structure of viriditoxin 38. Both SC-28763 40 and SC-30532 41 inhibited the anaerobic organisms Clostridium perfringens (MIC 1-16 µg/mL) and Trichomonas vaginalis (MIC 1 µg/mL). SC-28763 40 also showed modest activity against two strains of Candida albicans (MIC 50-75 µg/mL), the plant pathogen Verticillium albo-atrum (MIC 50 µg/mL) and the bacterium Staphylococcus aureus (MIC 50 µg/mL). Viriditoxin (SC-28762) 38 showed little or no activity against these microorganisms.

Monapinones A-E 42-46 and their co-occurring homo- and heterodimers the dinapinones 47-56 were identified as part of a program screening microbial metabolites for inhibitors of triacylglycerol (TG) synthesis using Chinese hamster ovary-K1 (CHO-K1) cells. Isolated from the culture broth of Talaromyces pinophilus (originally named Penicillium pinophilum) FKI-3864, the monapinones 42-46 were only produced when the production medium was supplemented with seawater. The monapinones exhibited weak TG inhibition, whereas dinapinones A2 48 and AB2 50 showed the most potent activities having IC₅₀ values of 0.65 µM and 1.17 µM, respectively. Interestingly, whilst the IC₅₀ values of the individual atropisomers dinapinone A1 47 and A2 48 were >12 µM and 0.65 µM, respectively, a 1:1 mixture of 47 and 48 had an IC₅₀ value of 0.054 µM. The relative stereochemistry of the hydroxylated C-3 side-chains in 42-56 was established from detailed analysis of ¹H NMR coupling constants and ROESY experiments whilst the axial configuration was assigned based on the observed Cotton effects in the CD spectra. The 3S configurations of 42 and 43 were deduced by comparison of their respective CD spectra with (R)-semivioxanthin 20. The in vitro dimerization of 42 using the lysate prepared from P. pinophilum FKI-3864 gave 47 and 48, establishing their absolute stereochemistries.

[[STRUCTURES 42-56 TO BE INSERTED HERE]]
macrophages, the suppression not being due to direct scavenging of NO.

Haemoventosin 59 is a red pigment isolated in 1971 from the lichen Ophioparma ventosa. The initially proposed furanonoaphthoquinone structure for haemoventosin was subsequently revised, twice, most recently to the quinonoid δ-lactone formulation. The structure evolved from extensive NMR experiments with the monoacetyl derivative of haemoventosin. The (S)-stereochemistry at C-3 in 59 was proposed after comparison of the circular dichroism spectrum of 1-O-acetylhaemoventosin with that of (R)-10-O-acetylsemixanthomegnin. The spectra were close to being mirror images of one another and therefore haemoventosin should possess the (S)-stereochemistry at C-3.

The dimeric pyranonaphthoquinone xanthomegnin 60, first isolated from the mould Trichophyton megnini and subsequently from T. rubrum and T. violaceum, occurs in many fungi including Nannizzia cajetani, Microsporium cookei, and various Penicillium and Aspergillus species. The linear structure of xanthomegnin superseded an earlier angular formulation in 1978 on the basis of 13C NMR experiments and was later confirmed by synthesis from natural semivioxanthin in Penicillium citreo-viride. The CD spectrum of xanthomegnin isolated from P. citreo-viride, along with the 1H NMR spectrum that shows splitting of the hydroxyl signals, indicates that 60 exists as a mixture of atropisomers. Xanthomegnin is a potent inhibitor of oxidative phosphorylation, it inhibits the growth of Bacillus subtilus and is a genotoxic carcinogen.

Viomellein 64 was first isolated from Aspergillus sulphureus and the structure was established by spectroscopic analysis and comparison with xanthomegnin. Oxidative degradation of 64 and comparison of the CD spectrum of the resulting isocoumarin with that of (R)-mellein established the (R) absolute configuration for 64. Viomellein 64 has since been found in A. ochraceus, A. melleus, Penicillium viridicatum, P. cyclopium and also in Nannizzia cajetani, where it co-occurs with 3',4'-dehydroviomellein, xanthomegnin 60, 3,4-dehydroxanthomegnin 66 and 3,4,3',4'-bisdehydroxanthomegnin.
Rubrosulphin 68 and viopurpurin 69 are co-metabolites in Aspergillus sulphureus, A. melleus and Penicillium viridicatum, with viopurpurin 69 also being obtained from Trichophyton violaceum. The structure of rubrosulphin 68 was established by spectroscopic analysis and confirmed by conversion of viomellein 64 to rubrosulphin 68 on heating in acetone in the presence of potassium carbonate.

The green colour of wood infected with the fungus Chlorociboria aeruginosa is due to the presence of the extended quinone xylindein 70. A process for artificially colouring wood with C. aeruginosa was patented and oak stained in this way was used for making "Tunbridge ware", a special kind of marquetry. The commercial application of wood stained by Chlorociboria sp. is still an area of active interest.

Over one hundred years ago Liebermann obtained the pigment in crystalline form for the first time. Structural studies of xylindein 70 were severely hampered by its extremely low solubility in all common organic solvents. In the 1920's Kogl made the first advances in the structure elucidation of xylindein and the complete structure was elucidated, independently, by Todd and co-workers and by Edwards and Kale. The methods relied on derivatization and degradative studies. Recently, X-ray crystallographic analysis of a bis para-bromobenzyl derivative of the natural product established that xylindein 70 itself has (3S,3'S) chirality.

2.4 Naphthopyranones coupled to non-naphthopyranones

As can been seen from the examples discussed above, both homodimeric and heterodimeric naphthopyranones are commonly encountered. In contrast, only a few naphthopyranones that are coupled to non-naphthopyranone aromatic systems have been isolated.

The naphthoquinone-coupled naphthopyranones xanthoviridicatin D 71 and G 72 were isolated, along with the previously known metabolites xanthomegnin 60, viomellein 64, rubrosulphin 68, and viopurpurin 69, from the mould Penicillium viridicatum. Xanthoviridicatin D 71 differs from viomellein 64 by the absence of a lactone ring in the naphthoquinone moiety. Similarly, xanthoviridicatin G 72, that contains an additional ether linkage, differs from the co-metabolite rubrosulphin 68 by the absence of one of the lactone rings. Conversion of xanthoviridicatin D to xanthoviridicatin G occurred upon heating in the presence of base in a manner similar to the formation of rubrosulphin 68 from viomellein 64 under these conditions. Later, the isomeric xanthoviridicatins E 73 and F 74 were isolated, through a bioassay-directed isolation protocol, from the fermentation broth of an endophytic strain of Penicillium chrysogenum. Xanthoviridicatins E 73 and F 74
inhibited the cleavage reaction of HIV-1 integrase with IC\textsubscript{50} values of 6 µM and 5 µM, respectively. Xanthoviridicatin E \textit{73} and F \textit{74} differ from xanthoviridicatin D \textit{71} by having further unsaturation of the lactone ring (i.e. incorporate a toralactone 1 moiety) and additional substitution on the naphthoquinone.

From the culture broth of \textit{Penicillium radicum} strain FKI-3765-2 were isolated the biaryl compounds xanthoradones A-C \textit{75}-\textit{77}.

Interestingly, \textit{75} and \textit{76} possess the dihydronaphthopyranone moiety exhibited by xanthoviridicatin D \textit{71} coupled to the trisubstituted naphthoquinone moiety contained in xanthoviridicatin E \textit{73} and F \textit{74}. However, the biaryl bond in xanthoradones A and B is between C-8 of the dihydronaphthopyranone and C-6' of the naphthoquinone, whereas in the xanthoviridicatin E and F the naphthoquinone moiety connects at C-3'. Xanthoradone C \textit{77} is isomeric to xanthoviridicatin E \textit{73}, differing only by the location of the biaryl bond at C-6' on the naphthoquinone. The nature of the Cotton effect in the CD spectra of xanthoradones A-C is similar to (\textit{M})-vioxanthin \textit{26}, suggesting \textit{75}-\textit{77} also have biaryl axes with (\textit{M})-configuration. Xanthoradones A and B exhibit moderate anti-MRSA activities, having an MIC of 4.0 µg/mL and 2.0 µg/mL, respectively. In combination with the β-lactam antibiotic imipenem, xanthoradones A-C \textit{75}-\textit{77} reduce the MIC of imipenem from 16 to 0.060, 0.030 and 0.50 µg/mL, respectively, suggesting unsaturation in the lactone is unfavourable for potentiating the activity of imipenem against the MRSA K-24 strain. Xanthoradones A \textit{75} and B \textit{76} show cytotoxicity on Jurkat cells with IC\textsubscript{50} values of 23.2 µg/mL and 2.6 µg/mL, respectively.

Cytosporacin \textit{78} was isolated from the fermentation broth of the fungus \textit{Cytospora rhizophorae} ATCC38475 and shown to contain a naphthopyranone linked to an isochromandione moiety. The structure \textit{78}, including the presence of a C-6/C-15 linkage, was established by extensive NMR analysis that also indicated the presence of two rotamers. Upon separation the individual components rapidly re-equilibrated to the original 6:5 mixture of rotamers. With the two aryl systems in \textit{78} lying perpendicular to each other, the relative stereochemistry of the two distant asymmetric centres could be established by the observation of an NOE between H-16 and both H-4 and H-5 for one rotamer, and an NOE between H-5 and both H-16 and H-21 for the other rotamer. A \textsuperscript{13}C-labelling experiment established the polyketide biosynthetic origin of \textit{78}. Cytosporacin \textit{78} showed modest \textit{in vitro} activity against Gram-positive bacteria.

Torosaol II \textit{79} represents an unusual heterodimeric system that consists of a naphthopyranone half that closely resembles cassialactone \textit{19} (with additional methylation at C-8) and the other component being a dihydroanthracenone (7-methyltorosachrysone). The anisotropic effect resulting from the biaryl bond being located between C-5 and C-10' in \textit{79} results in an upfield shift of signals in the \textsuperscript{1}H NMR spectrum of \textit{79} in comparison to the monomer cassialactone \textit{19}. The effect is particularly evident in signals from the C-4 methylene and C-6 aromatic protons, as is observed in related biaryl systems. Isolated from the roots of \textit{Cassia torosa} along with a number of other dihydroanthracenones, torosaol II \textit{79} is reported to have an ED\textsubscript{50} of 5.5 µg/mL against an \textit{in vitro} KB cell line.
2.5 Naphthopyranones with extended ring systems

Natural products that possess the tricyclic naphthopyranone core structure are the focus of the present discussion, however, it is worth noting that the naphthopyranone moiety can also be incorporated within an extended ring system. In such natural products, ring fusion may be present on the lactone ring or as an extension of the aromatic system, as can be seen by the examples that follow.

In contrast to the relatively widespread occurrence of natural naphthopyranones, anthrapyranones are rare natural products. Thermorubin, produced by the bacterium *Thermoactinomyces antibioticus*, possesses strong antibiotic properties resulting from blocking the initiation phase of bacterial protein synthesis. The structure was firmly established by single crystal X-ray analysis, and numerous derivatives of thermorubin have been prepared and used for structure activity studies. Thermorubin shows activity against several bacteria including *Staphylococcus aureus* (MIC 0.006 µg/mL) and *Streptococcus pneumoniae* (MIC 0.05 µg/mL). A recently obtained co-crystal structure of thermorubin complexed with the 70S ribosome from *Thermus thermophilus* shows conformational changes in the ribosome that are likely to be responsible for the activity. Thermorubin inhibits aldose reductase in a dose-dependent manner with IC₅₀ values of 1.3 nM and 54 nM on rat lens and human aldose reductases, respectively, whilst not inhibiting other reductases. The use of thermorubin and its derivatives as aldose reductase inhibitors for the prevention and treatment of complications resulting from diabetes has been patented.

Several toadstools from the subgenus *Leprocybe* of *Cortinarius* contain anhydrolepolutein as a trace constituent, whilst from the Australian toadstool *Dermocybe sanguinea* (sensu Cleland) have been isolated the quinones dermolactone and 5-hydroxydermolactone. The stereochemistry of dermolactone was established by synthesis of the (S)-enantiomer and direct comparison of the chiroptical and spectroscopic properties with the natural material. Interestingly, this comparison established that the natural material is an anisochiral mixture in which the (S)-enantiomer predominates to the extent of 64 to 36%.

The plant *Swertia mileensis*, used in traditional Chinese medicine to treat viral hepatitis, has been the subject of investigations to identify the active anti-hepatitis B virus (HBV) compounds. As a result of these studies swerilactone E was isolated and shown to possess significant activity, based on an anti-HBV assay on the Hep G 2.2.15 cell line in vitro, inhibiting the secretion of hepatitis B virus surface antigen (HBsAg) (IC₅₀ 0.22 mM) and hepatitis B virus e antigen (HBeAg) (IC₅₀ 0.52 mM). Accompanying swerilactone E in *S. mileensis* was the 6,7-dihydro derivative that showed lower activity. The lack of oxygenation and the additional 8,9-fused lactone ring suggests a novel biosynthetic origin. The biogenesis of more complex members of the swerilactone family has been proposed to involve secoiridoid intermediates.

Polycyclic xanthone IB-00208 was isolated from the culture broth of a marine-derived species of *Actinomadura*. The relative stereochemistry of the sugar moiety, but not C-3 of the pyrone ring, could be established by spectroscopic analysis. Along with good activity against Gram-positive organisms (MIC values of 0.09-1.4 nM), IB-00208 exhibits potent cytotoxic activity (MIC 1 nM) against both human (A-549, HT-29 and SK-MEL-28) and murine (P388D1) tumor cell lines.

Chartreusin was first isolated in 1953 from the culture broth of soil-derived *Streptomyces chartreusis* and shown to have antibiotic activity, the complete structure being...
elucidated in 1960.\textsuperscript{125} Chartreusin \textbf{86} was shown to exhibit excellent antitumor activity against murine P388, L1210 leukemia and B16 melanoma cell lines, however its rapid elimination through the bile and slow gastrointestinal absorption limits its clinical potential.\textsuperscript{126} In the search for related compounds with greater therapeutic potential, an unidentified actinomycete strain \textit{J907w21} isolated from a soil sample of El Salvador was shown to produce elsamicins A \textbf{88} and B \textbf{89}.\textsuperscript{127} Elsamicin A \textbf{88} exhibited greater antitumour activity than chartreusin \textbf{86}, attributed to the greater solubility resulting from the presence of an amino sugar, whereas elsamicin B \textbf{89} was nearly inactive. Further related compounds have been isolated, including 3''-demethylchartreusin \textbf{87} from \textit{Streptomyces chartreusis} that shows similar antitumor activity to chartreusin \textbf{86},\textsuperscript{128} D329C \textbf{90} from a mutant of \textit{S. chartreusis},\textsuperscript{129} and from marine sediment \textit{Streptomyces} isolates the 2''- and 4''-monoacetates of chartreusin \textbf{86}.\textsuperscript{130} Using the combined techniques of biosynthetic engineering and mutasynthesis has given rise to further analogues of potential therapeutic value that are not available readily through synthetic protocols alone.\textsuperscript{131} The significant antitumor effects of chartreusin \textbf{86} and related structures principally involves binding to DNA\textsuperscript{132} but can also involve DNA-cleavage through the production of free radicals.\textsuperscript{133} A wealth of pharmacological data has been obtained on chartreusin \textbf{86}, elsamicins A \textbf{88} and B \textbf{89} and related compounds\textsuperscript{134} allowing a number of members of this family of anti-cancer agents to proceed to phase II clinical trials.\textsuperscript{135}

More recently the monomeric furan-fused naphthopyranone lasionectrin \textbf{93} has been isolated from a species of \textit{Lasionectria} obtained from forest fern leaf litter in Equatorial Guinea.\textsuperscript{137} Bioassay-guided fractionation led to the isolation of \textbf{93} that exhibits an \textit{IC}_{50} value of 11.0 \textmu M against \textit{Plasmodium falciparum Pf3D7}. The relative stereochemistry depicted in \textbf{93} was determined by NMR analysis.

From the roots of \textit{Rubia cordifolia} was isolated rubicordifolin \textbf{94} that was shown to possess significant cytotoxic activity \textit{in vitro} against Chinese hamster lung (V-79) cells (\textit{IC}_{50} 4.7 \textmu g/mL), human nasopharynx carcinoma (KB) cells (\textit{IC}_{50} 1.2 \textmu g/mL) and P388 lymphocytoid leukemia cells (\textit{IC}_{50} 2.9 \textmu g/mL), and \textit{in vivo} antitumor activity against Sarcoma 180 ascites in mice.\textsuperscript{138} The relative stereochemistry depicted in \textbf{94} was established by NOE analysis of a sample of rubicordifolin prepared during later synthetic studies that also established a likely biosynthetic origin of rubicordifolin \textbf{94}, which is found naturally as a racemate.\textsuperscript{139}

Lichenicolins A \textbf{91} and B \textbf{92} were isolated from an unidentified lichenicolous fungus, strain \textit{LL}-RB0668.\textsuperscript{136} The structures were determined by NMR analysis with the \textit{13}C NMR shift data for lichenicolin A \textbf{91} being almost identical to semivioxanthin \textbf{20} in the aromatic region except for the C-8 signal, indicating a C-8/C-8' linkage between the equivalent halves. The location of the biaryl linkage was further established from the ROESY spectrum that was also used to establish the \textit{cis} ring junction between the lactone (\textit{\alpha}-pyrone) and furan rings and the relative orientation of the furan methyl substituent. Lichenicolin B \textbf{92}, obtained as a minor isolation product, has undergone oxidation of one of the naphthalene moieties to give the unsymmetrical dimer. Interestingly, lichenicolin A showed moderate to good antimicrobial activity against a range of Gram-positive and Gram-negative bacteria, whereas lichenicolin B was found to be inactive.
2.6 Engineered naphthopyranones

Whilst a number of the naphthopyranones discussed thus far have been isolated after modification of the growing media conditions in which the source organism is cultivated, the emerging area of metabolic engineering has opened up new avenues for the isolation of novel secondary metabolites. In this regard the ability to manipulate polyketide synthase (PKS) gene clusters allows mutants to be produced in a deliberate and controlled manner. By inactivating genes responsible for a specific step in the biosynthesis of polyketide metabolites, intermediates (or shunt products) can be isolated that both give information about biosynthetic pathways and may also have their own beneficial properties. A number of novel naphthopyranones have been isolated using such processes.

The C-1 methyl substituent of chartreusin is derived biosynthetically from an acetyl starter unit being incorporated into the chartreusin PKS. In order to produce the chartreusin analogue homochartreusin, Hertweck constructed a cha PKS mutant complimented with genes coding for aclacinomycin biosynthesis that produce a polyketide primed with propionate. As a result, an ethyl substituent is incorporated into homochartreusin at C-1. Homochartreusin and chartreusin showed similar antitumoral and cytostatic activity in Kw562 and HeLa tumour cell lines, whereas synthetic norchartreusin that lacks an alkyl substituent at C-1 showed significantly lower activity.

In order to gain further insight into how the crucial 5,6-spiroacetal moiety contained in the rubromycin family of compounds is formed biosynthetically, a series of gene-deletion variants of the griseorhodin A gene cluster were constructed. As a result, the biosynthetic intermediate lenticulone was isolated and shown to possess a naphthopyranone moiety in which the C-ring forms part of a 6,6-spiroketal. The key modification in transforming lenticulone to griseorhodin A is excision of the C-1 carbonyl group in 98, possibly involving a decarboxylative process, to form a 5,6-spiroketal system. Lenticulone showed similar bioactivity to griseorhodin A in antiproliferative assays using K-562 cells (GI<sub>50</sub> 0.035 µmol/mL), cytotoxicity against HeLa cells (CC<sub>50</sub> 0.090 µmol/mL) and antibacterial activity against Staphylococcus carnosus and Bacillus subtilis.

By deconstruction and reassembly of the iterative polyketide synthase (IPKS) responsible for biosynthesis of aflatoxin B<sub>1</sub>, Townsend showed that production of the usual anthraquinone biosynthetic intermediate could be attenuated in favour of the new shunt product norpyrone. The production of norpyrone occurred in constructed enzymes in which the thioesterase/Claisen cyclase (TE/CLC) domain was not present, favouring spontaneous product release via pyrone formation rather than intramolecular Claisen reaction to form an anthrone product.

By cloning the gene cluster responsible for the biosynthesis of the benastatins and subsequent deletion of the benQ gene, a new group of novel metabolites were formed. Amongst these were benastatins F and H that were accompanied by their respective 6,7-dihydro analogues. Benastatin H showed antiproliferative effects against the L-929 mouse fibroblast cell line (GI<sub>50</sub> 22.4 µg/mL) and human leukemia cell line K-562 (GI<sub>50</sub> 24.7 µg/mL) and cytotoxicity against HeLa cells (GI<sub>50</sub> 22.8 µg/mL). The benQ gene determines starter unit selection during biosynthesis of the benastatins and, as such, it was established that the products 96 and 97 likely arise by priming with a butyrate rather than the usual hexanoate primer that is used in production of benastatins from a non-mutated Streptomyces sp.
Polyketide synthase CTB1 from *Cercospora nicotianae* is responsible for the first assembly steps towards the biosynthesis of the perylenequinone cercosporin. A reconstructed CTB1 set of domains was shown to produce *nor*-toralactone 100 as the major product.\(^1\) The thioesterase (TE) domain of CTB1 was shown to accelerate pyrone 100 formation possibly by promoting keto/enol tautomerization in the active site leading to release of 100 through enol-lactonization. The starter unit acetyltransferase (SAT) domain of CTB1 initiates polyketide extension by utilizing acetylwCoA selectively, leading to generation of *nor*-toralactone 100 via heptaketide formation. By using 'domain-swapped' CTB1 that incorporates a hexanoyl starter unit an analogue of *nor*-toralactone 100 was produced and proposed to be the naphthopyranone 101.\(^2\) As evidenced by the examples outlined above, the ability to engineer the enzymes responsible for polyketide biosynthesis provides exciting opportunities to custom design new polyketide-derived products, including novel naphthopyranones.

3. Biosynthesis

The methods that have been used to investigate the biosynthesis of naphthopyranones somewhat reflect the techniques available at the time of their isolation, developing in their complexity in parallel with advances in the field of polyketide biosynthesis.\(^3\) Early investigations principally relied on isotopic labelling experiments, whilst more recent studies have utilized engineered enzymes to probe biosynthetic pathways, including the type II PKS genes and enzymes responsible for the synthesis of aromatic polyketides.\(^4\)

The biosynthesis of xanthomegnin 60 has been studied by feeding sodium [1-\(^{13}\)C]- and [1,2-\(^{13}\)C\(_2\)]-acetate to *Aspergillus melleus*.\(^5\) The \(^{13}\)C-enriched xanthomegnin 60 so obtained (Scheme 1) showed enhancement of seven of the fourteen carbon signals in the \(^{13}\)C NMR spectrum consistent with the homodimeric structure, the enhanced signals corresponding to alternate carbons as would be expected for a regular heptaketide 102 assembly. Labelled viomellein 64 was also isolated and showed it to be derived from fourteen intact acetate units.

![Scheme 1: Incorporation of sodium [1,\(^{13}\)C]-acetate into xanthomegnin 60.](image1.png)

The process by which formation of dimeric naphthopyranones takes place is itself an interesting aspect of the biosynthesis of these natural products. To investigate this Müller prepared the \(^{13}\)C-labelled naphthopyranones \((R)-[1,\(^{13}\)C]-25 and \((R)-[O\(^{13}\)CH\(_3\)]-20 (Scheme 2).\(^6\) The \(^{13}\)C-labelled substrates 25 and 20 were then used in feeding experiments with *Penicillium citreo-viride* resulting in the isolation of double-labelled vioxanthin 26 in both cases. This observation established that C-7 \(O\)-methylation of 25 to produce semivioxanthin 20 takes place before regio- and atroposelective phenolic coupling to form the C-8/C-8\(^{\prime}\) dimer, vioxanthin 26.

![Scheme 2: Incorporation of \(^{13}\)C-labelled substrates 20 and 25 into vioxanthin 26.](image2.png)

It would be anticipated that other naphthopyranones originate from a similar folding pattern during their biosynthesis to that elucidated for xanthomegnin 60 and vioxanthin 26. Direct evidence to confirm this proposition has been obtained for 7-de-O-methylsemivioxanthin 25 (Figure 3).\(^7\) In the case of cytosporacin 78, incorporation of [1,2-\(^{13}\)C\(_2\)]-acetate confirmed that the naphthopyranone moiety is formed.
from a heptaketide in the usual fashion and the isochromandione is derived from an octaketide.\textsuperscript{108} It is pertinent to note that for the monapinones 42-46, and their corresponding homo- and heterodimers, the C-3 alkyl substituent consists of a C\textsubscript{7} or C\textsubscript{9} chain length, consistent with incorporation of an additional acetate group for some members. A similar observation can be made for the lichenicolins A 91 and B 92 that have a C\textsubscript{3} unit at C-3, whereas the monomeric lasionectrin 93 that bears a close structural resemblance but is isolated from a different organism has a C\textsubscript{4} unit at C-3.

Figure 3: Incorporation of labelled acetate ([1-\textsuperscript{13}C] or [1,2-\textsuperscript{13}C\textsubscript{2}]) into naphthopyranones.

Dermolactone 82 was shown to originate from a nonaketide precursor 103 (Scheme 3) by incorporation of [1-\textsuperscript{13}C]-acetate into the product 82.\textsuperscript{119} Whilst dermochrysone 106, a co-metabolite of dermolactone 82 in Dermocybe sanguinea (sensu Cleland), has (S) stereochemistry, dermochrysonol 107 occurs as a mixture of diastereoisomers epimeric at the secondary alcohol stereocentre. This is consistent with the isolation of dermolactone 82 as an unequal mixture of enantiomers, suggesting that reduction of 104 to 105 occurs non-stereoselectively.

Scheme 3: Incorporation of [1-\textsuperscript{13}C]-acetate and proposed biosynthesis of dermolactone 82.

A number of naphthopyranones require some modification to the regular linear folding pattern and condensation process seen in the examples above. The biosynthesis of thermorubin 80 was postulated, amongst other possibilities, to be based on the combination of an undecaketide and salicylic acid.\textsuperscript{113} Direct evidence for the biosynthetic origin of thermorubin 80 was obtained by Aragozzini and co-workers by feeding Thermoactinomyces vulgaris with [1-\textsuperscript{13}C]-, [2-\textsuperscript{13}C]-, and [1,2-\textsuperscript{13}C\textsubscript{2}]-acetate.\textsuperscript{149} Incorporation of the labelled acetate (Scheme 4) showed that the A-D rings in 80 originate from an undecaketide chain 109, the lactone being formed by a Woodward oxidative fission of ring D in the proposed intermediate 110. Labelled salicylic acid 108 was also incorporated into 80 (73% isotopic enrichment of C-3'), accounting for the origin of the additional phenyl ring, salicylic acid being available naturally via the shikimate pathway.
Early investigations into the biosynthesis of chartreusin 86 showed that the aglycone chartarin 114 is derived from acetate, however, clearly the end product 114 (Scheme 5) is not formed directly from folding of a polyketide intermediate and has instead undergone rearrangement. Feeding experiments using $[1-^{13}\text{C}]$, $[1,2-^{13}\text{C}_2]$-acetate and $^{13}\text{C}$-labelled salicylic acid 108 into thermorubin 80 identified the origin of each carbon in the skeleton of 114 and led to the initial proposal that chartreusin 86 is formed via an angularly folded undecaketide intermediate 115 (Path B). A revised biosynthesis of chartreusin 86 involving the more conventional linearly fused decaketide 111 (Path A) has been proposed after a detailed analysis, by pathway dissection, of the genes involved. An engineered mutant lacking the chaZ gene produces the known anthracyclic polyketide resomycin C 112 in lieu of chartreusin 86. Resomycin C 112 may undergo C-5/C-5a bond cleavage followed by bond formation between C-5/C-11 to give angular intermediate 113. Loss of 'C-6', possibly by oxidation, and lactone formation would give chartarin 114.

A more detailed knowledge of the mechanisms involved in the biosynthesis of polyketides, including those leading to a selection of naphthopyranones, is beginning to emerge through the use of gene manipulation. As a representative example, nor-toralactone 100 is formed through the involvement of a non-reducing polyketide synthase (NR-PKS). Such systems are typically characterised by a set of six catalytic domains (Scheme 6); the starter unit acyltransferase (SAT), ketoacyl synthase (KS), malonyl acyltransferase (MAT), product template (PT), acyl-carrier protein (ACP) and thioesterase (TE) domains. By constructing the CTB1 domain responsible for the first assembly steps of cercosporin, nor-toralactone 100 could be isolated. The biosynthesis proceeds along a typical pathway of starter unit selection (controlled by SAT), chain extension, regioselective aldol cyclization controlled by the PT domain and product release from TE. Product release from the enzyme may occur by hydrolysis to form carboxylic acid 116 followed by hemiketal formation and dehydration to give pyrone 100. However, selective labelling of the polyketide starter unit by use of $[^{18}\text{O}]$acetyl-CoA led to formation of $^{18}\text{O}$-labelled nor-toralactone 100 indicating that product release occurs by TE-catalysed pyrone formation (enol-lactonization).
In the case of nor-toralactone 100 the TE domain is crucial to catalyse product release and resultant pyrone formation.\textsuperscript{144} In other systems, such as the constructed NR-PKS responsible for the synthesis of aflatoxin, the absence of the thioesterase/Clausen cyclase (TE/CLC) domain can lead to pyrone formation as a shunt product in lieu of the usual potential and could conceivably lead to the ability to undertake de novo synthesis of polyketide products using designed enzymes.

4. Synthesis

4.1 Semisynthesis

Despite the common occurrence of naphthopyranones as natural products the number of reported syntheses of members of this group is limited. A selection of naphthopyranones have been obtained by semisynthesis whereby one naphthopyranone, isolated from a natural source, has been converted to a closely related natural product. Examples of this are prevalent in the early investigations of quinonoid-containing naphthopyranones. In particular, studies by Zeeck and co-workers in 1979 were crucial in verifying the structure of xanthomegnin 60 by its synthesis from a co-metabolite.\textsuperscript{38} Thus, oxidation using Fremy’s salt of (R)-semivioxanthin 20 (Scheme 7), obtained from Penicillium citreo-viride, gave semixanthomegnin 61 that was not itself identified as a natural product until 2008. Demethylation of 61 followed by dimerization using persulphate gave luteosporin 62 that upon methylation delivered xanthomegnin 60 that was shown to be identical to natural xanthomegnin. Xanthomegnin 60 has itself been converted, in low yield, to plastatin 63 by treatment with ammonium hydroxide and relactonization.\textsuperscript{93} Finally, viomellein 64 obtained from Aspergillus sulphureus was shown to undergo conversion to rubrosulphin 68 upon heating with potassium carbonate, thus supporting the assigned structures of these co-metabolites.\textsuperscript{88} In combination, these studies have proved particularly useful in providing confirmation of the structural relationships between this group of natural products.

The majority of total syntheses of naphthopyranones that have appeared employ either a biomimetic strategy, involve an annulation strategy based on a tandem Michael addition-Dieckmann condensation reaction or use a Diels-Alder cycloadDITION approach. The use of these methods in the total synthesis of naphthopyranone natural products will be discussed in the following sections.

4.2 Biomimetic approaches

The first synthesis of (±)-semivioxanthin 20 involved the intramolecular condensation of a synthetic polyketide.\textsuperscript{152} It began from ketal-protected diethyl β-oxoglutarate 117 that was reacted with two equivalents of the dianion from tert-butyl acetoacetate to give the polyketide 118 (Scheme 8). With calcium diacetate intermediate 119 suffered a series of intramolecular condensations to give 120. A series of protection and deprotection steps lead to the diester 120, which was subjected to a selective Clausen reaction with the enolate from tert-butyl acetoacetate to give the keto-ester 121. Removal of the tert-butyl ester in 121, decarboxylation and final reduction gave (±)-semivioxanthin 20 in a yield of 4% over the ten steps.
During synthetic studies of Rubiaceae natural products the facile preparation of rubicordifolin 94 was achieved by treating the postulated biosynthetic intermediate vinyl naphthoquinone 127 with phenylboronic acid (Scheme 10). Under these conditions naphthoquinone 127 undergoes reversible cyclization to form ortho-quinone methide 128, calculations showing this to be a viable process. Naphthofuran 129, also formed from 127 via cyclization and dehydration, then undergoes hetero-Diels-Alder cycloaddition in combination with 128 to lead ultimately to rubicordifolin 94. Favourable π-stacking in the transition state during cycloaddition and the steric bulk of the hydroxyisopropyl group in 128 appear to control the diastereoselectivity of the reaction. Prenylated naphthoquinones related to 127 are found in Rubiaceae sp. and, as such, this pathway may account for the occurrence of rubicordifolin 94 naturally as a racemate.

[[SCHEME 10 TO BE INSERTED HERE]]

4.3 Annulation approaches

The Staunton-Weinreb annulation procedure is ideally suited to the rapid construction of naphthopyranones and has thus become a standard method for the preparation of this class of compounds, both in the synthesis of naturally occurring naphthopyranones and for the preparation of versatile naphthopyranone intermediates en route to other structure classes. Examples of the use of this approach for the synthesis of naturally occurring naphthopyranones will be discussed in this section.

Developed by Staunton for the synthesis of toralactone 1, the Staunton-Weinreb annulation involves the generation of an ortho-toluate anion that is able to undergo a tandem Michael addition-Dieckmann condensation with α,β-unsaturated lactones. In the case of toralactone 1 the stannylated orsellinate 130 and the lactone 131 were used (Scheme 11). Firstly, deprotonation of the orsellinate 122 with LDA and trapping of the resultant anion gave the trimethylstannyl derivative 130. Subsequent treatment of 130 with n-butyllithium generated the anion of 122 that underwent a tandem Michael-Dieckmann reaction with the α,β-unsaturated lactone 131 to give toralactone-9-O-methyl ether 132. Direct reaction of the lactone 131 with the anion of 122 generated from LDA gave the same product 132 but in lower yield. Subsequently, the ether 132 has been converted to toralactone 1 by selective ether cleavage using boron tribromide. More recently, toralactone 1 prepared in this way was then converted to cassiaside C 3 and cassiaside C 4 by selective glycosylation of the C-9 hydroxy group.
A concise synthesis of (±)-semivioxanthin 20, reported by Deshpande, involves a tandem Michael-Dieckmann reaction between orsellinate 140 and lactone 141 (Scheme 13). Removal of the protecting group gave semivioxanthin 20 that, upon oxidation, delivered semixanthomegnin 61. More recently this approach was adapted to a stereoselective synthesis of both (R)- and (S)-semivioxanthin. For the synthesis of (R)-semivioxanthin 20 (Scheme 14), enzymatic reduction of tert-butyl 3,5-dioxohexanoate afforded tert-butyl (R)-5-hydroxy-3-oxohexanoate 142 in 99.4% e.e. The ester 142 was subsequently lactonized and then converted to the enol methyl ether (R)-141. Tandem Michael-Dieckmann reaction between the orsellinate 143 and the lactone (R)-141 followed by protecting group removal then gave (R)-semivioxanthin 20. (S)-Semivioxanthin 24 (97% e.e.) was prepared similarly beginning from tert-butyl (S)-5-hydroxy-3-oxohexanoate ent-142, which was processed in a similar manner to the (R)-enantiomer. The 13C-labelled naphthopyranones (R)-(1-13C)-25 and (R)-[O13CH3]-20 (Scheme 2), used to investigate the biosynthesis of vioxanthin 26 in Penicillium citreo-viride, were prepared using a similar strategy to that depicted in Scheme 14.21

![SCHEME 13 TO BE INSERTED HERE]]

![SCHEME 14 TO BE INSERTED HERE]]

Since a variety of readily available substituted orsellinates have been shown to react with α,β-unsaturated lactone electrophiles, the ability to access the chiral lactone electrophilic component efficiently is an important consideration in applying the above strategy to a range of naphthopyranone targets. To this end, it has been shown that lactones such as (R)-141, 147 and 148 are readily available in two steps from terminal epoxides 144-146 (Scheme 15). The simplest of these lactones, methyl substituted lactone (R)-141, underwent Staunton-Weinreb annulation with orsellinate 149 to form 150 with final deprotection delivering (R)-semivioxanthin 20 in 17% yield over four steps from (R)-propylene oxide 144. Beginning with racemic propyl oxirane 145 the C-3 propyl-substituted naphthopyranone 152 was also available in four steps (17% yield). Naphthopyranone 152 constitutes one half of the symmetrical dimers talarodexines A 34 and B 35 and also a potential precursor to xylindein 70. For the first total synthesis of (S)-semiviriditoxin 36, chiral epoxide 146 was used to prepare lactone 148 that underwent Staunton-Weinreb annulation to generate naphthopyranone 153. Straightforward conversion to (S)-semiviriditoxin 36 was then effected. Spectroscopic comparison of synthetic (R)-semivioxanthin 20, synthetic (S)-semiviriditoxin 36 and the reported data for naturally occurring 36 confirmed the (S)-stereochemistry of semiviriditoxin 36 from the fungus Paecilomyces variotii.161

![SCHEME 15 TO BE INSERTED HERE]]

With numerous naphthopyranone natural products occurring as dimers, synthetic methods for accessing such structures have been developed. Two general methods can be envisaged for the preparation of dimeric systems using the Staunton-Weinreb annulation, firstly, oxidative biaryl coupling of a monomeric naphthopyranone precursor, and secondly, a double annulation of a bis-orsellinate precursor. Both of these methods have been applied to the synthesis of homodimeric naphthopyranones.

The enantioselective synthesis of both (M,R,R)- and (P,R,R)-vioxanthin 26 was undertaken by Müller as part of an investigation of the biosynthesis of biaryl secondary metabolites.21 For the synthesis of (P,R,R)-vioxanthin 26 (Scheme 16), atropisomerically pure bis-orsellinate (P)-154 was used. A double Staunton-Weinreb annulation with lactone (R)-141 followed by selective deprotection gave the dimeric naphthopyranone (P,R,R)-vioxanthin 26. Similarly, the atropisomer (M,R,R)-vioxanthin could be prepared from bis-orsellinate (M)-154. Access to both (M,R,R)- and (P,R,R)-vioxanthin 26 by synthesis was crucial for confidently assigning the absolute stereochemistry of natural vioxanthin 26.21

![SCHEME 16 TO BE INSERTED HERE]]

For the synthesis of viriditoxin 38, Shaw used a vanadium-catalyzed naphthopyranone dimerization procedure as the key step in forming the C-6/C-6’ homodimeric system (Scheme 17). In this approach the required naphthopyranone intermediate 157 was prepared by annulation between orsellinate 155 and lactone 156 followed by protecting group manipulation. The β-naphthol 157 then underwent oxidative coupling using VO(acac)2 to give the naphthopyranone dimer 158, with the remote stereocentres exerting diastereocontrol to produce the desired atropisomer (M)-158 (dr 76:24). Improved
atroposelectivity (dr 89:11) was obtained using a chiral BINOL-derived vanadium catalyst. A further five steps from 158 completed the first synthesis of viriditoxin 38 and established the absolute configuration of the natural product.

Scheme 17

This methodology has been further adapted to the synthesis of the dimeric naphthopyranones pigmentosin A 27 and talaroderxines A 34 and B 35 (Scheme 18). Thus, annulation between orsellinate 155 and the methyl-substituted lactone 159 leads, ultimately, to pigmentosin A 27. The corresponding propyl-substituted lactone 160 was reacted similarly giving rise to each of the talaroderxines A 34 and B 35. In the latter case the choice of chiral vanadium catalyst used for the phenolic coupling enabled effective control of the axial stereochemistry to deliver the desired atropisomeric product.

Scheme 18

The synthesis of chartarin 114, the aglycone of chartreusin 86, has been undertaken by a number of groups. An efficient synthesis of chartarin 114 from benzaldehyde 161 has been achieved by Mal and co-workers in which a Hauser-Kraus annulation between coumarin 162 and cyanophthalide 163 (Scheme 19) was used to rapidly assemble the benzophenanthroquinone core 164, final demethylation providing chartarin 114. A similar strategy was used by Hertweck to prepare chartarin 114 and the analogues 165-167 (Scheme 20). When synthetic chartarin 114 and 165-167 were added to the heterologous host Streptomyces albus, a chartreusin (cha) PKS null mutant not capable of producing chartarin 114, chartreusin 86 and analogues 168-170 were isolated successfully. Vinylchartreusin 168 showed slightly lower activity against K-562 and HeLa tumour cells when compared to chartreusin 86, however upon photoactivation a 12-fold increase in the activity of 168 was shown (GI₅₀ 0.6 µM) in a colon adenocarcinoma cell line (HT-29), resulting from [2 + 2] photo adduct formation between vinylchartreusin 168 and DNA. Notably, norchartreusin 169 showed a significant loss in cytotoxic activity, whereas chartreusin regioisomer 170 retained activity. As demonstrated in the preparation of chartreusin 86, the modern approach of linking chemical synthesis with biosynthesis (mutasynthesis) has vast potential for accessing analogues not readily obtainable either through total synthesis or derivatization of natural products.
4.4 Diels-Alder approaches

Whilst quinonoid-containing naphthopyranones have been prepared by oxidation of the corresponding naphthol, quinonoid compounds are also suited to synthesis directly using a Diels-Alder approach. To establish the stereochemistry of dermolactone 82, its stereoselective total synthesis was undertaken. The reaction sequence that leads to (S)-dermolactone 82 is summarized in Scheme 21.\(^1\)\(^2\) Thus, reaction between (S)-propylene oxide 144 and lithium acetylide gave (S)-pent-4-yn-2-ol that was silylated and carboxmethoxylated to afford the hexynoate ester 171. Reaction of the ester 171 with ketene dimethyl acetal then gave the highly functionalized chiral butadiene (S)-172. When diene (S)-172 was heated with 2-chloro-1,4-naphthoquinone 173, anthraquinone 174 was obtained regioselectively. Removal of the silyl protecting group under acidic conditions, lactonization and finally selective demethylation of the sterically most encumbered 12-O-methyl ether group afforded (S)-dermolactone 82 in high enantiomeric purity. The availability of enantiomerically pure (S)-dermolactone 82, obtained by synthesis, was crucial in identifying that natural dermolactone exists as an unequal mixture of enantiomers.

![Scheme 21](image)

The potential versatility of the highly functionalized diene 172 to be used in the synthesis of other lactone-fused aromatic systems was realized by its reaction with various dienophiles. Thus, the (R)-enantiomer of 172, prepared from (R)-propylene oxide in the same manner as described above for (S)-172, when heated with 2,5-dichlorobenzoquinone 175 formed naphthoquinone 176 (Scheme 22). Substitution using methoxide and lactone formation completed the first total synthesis of (R)-semixanthomegnin 61.\(^3\)\(^2\) The flexibility of this Diels-Alder approach was further demonstrated by the synthesis of ochratoxin-\(\alpha\) 178. In this case diene (R)-172 reacted effectively with the acetylenic dienophile methyl propiolate to give benzoate 177 that was further transformed to ochratoxin-\(\alpha\) 178, completing a formal synthesis of ochratoxin A 179.\(^4\)\(^5\) Ochratoxin A 179 co-occurs with xanthomegnin 60 in several Penicillium species, including those contaminating agricultural products,\(^7\) and is a nephrotoxin, immunosupressant, teratogen and carcinogen that is implicated in several human diseases.\(^6\)\(^6\) A similar Diels-Alder strategy to that shown in Schemes 21 and 22 has been explored for the synthesis of xylindine 70.\(^6\)\(^7\)

5. Conclusions

Scientific interest in the chemistry of naphthopyranone compounds has a long history extending back to the 19\(^{th}\) century when the blue-green pigment responsible for the natural staining of wood used for decorative purposes was being investigated, later the responsible agent being identified as xylindine 70. Over 130 years on from these early studies, state-of-the-art techniques have now demonstrated the possibility of constructing engineered polyketide synthases designed to produce novel naphthopyranones, such as norpyrone 99. Along the way naphthopyranones have been shown to occur in a range of organisms including fungi, bacteria, lichen and plants, with most examples showing some level of antibiotic, cytotoxic, antioxidant, immunoregulatory, antimalarial or antifungal activity, to name but a few of the diversity of bioactivities identified. Some members have shown significant potential for further development, such as analogues of chartreusin 86 that have proceeded to phase II clinical trials for the treatment of cancer. The preparation of naphthopyranones, either by semisynthesis or total synthesis, has played an important role in confirming structural details such as absolute stereochemistry in a number of naturally occurring naphthopyranones. The desire to develop new methods to enable efficient synthesis of these entities has expanded the broader art of organic synthesis, with recent developments in the area of mutasynthesis beginning to have a beneficial impact in the preparation of naphthopyranones and novel analogues otherwise not readily available through total synthesis. After a long history of investigation, new opportunities are now opening up for exploitation of naphthopyranones, particularly for their pharmaceutical potential.

6. Notes and references


49 For consistency, the (M) / (P) descriptors are used throughout this manuscript to designate axial stereochemistry. The descriptors (M) and (P) are equivalent to (R) and (S), respectively.


THE FOLLOWING GRAPHICS/SCHMES NEED TO
BE INSERTED INTO THE TEXT AT THE INDICATED
POSITIONS

[[STRUCTURES 1-16]]:
[[STRUCTURES 20-26]]:

[[STRUCTURES 30-32]]:

[[STRUCTURES 42-56]]:
[[SCHEME 6]]:

[[SCHEME 7]]:
[[SCHEME 8]]:

[[SCHEME 10]]:

[[SCHEME 13]]:

[[SCHEME 14]]:
[[SCHEME 15]]:

[[SCHEME 22]]: