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# METABOLITES FROM MARINE SPONGES OF THE GENUS *PLAKORTIS*

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**Abstract** – Metabolites from the *Plakortis* genus of sponges are reviewed, with major focus on *P. halichondrioides*, *P.simplex*, *P. angulospiculatus*, *P. lita*, *P. nigra*, *P. quasiamphiaster*, *P. zygompha*, and the closely related *Plakinastrella onkodes*. The structures, stereochemistry, pharmacological activity and selected syntheses of these metabolites are discussed. Peroxy containing, polyketide derived metabolites constitute a prevalent class of biologically potent metabolites from *Plakortis* species.

Recent decades have seen enormous interest in the metabolites from a wide variety of marine organisms. The existence of a large universe of structurally diverse compounds has been demonstrated, in the reviews, *Natural Product Reports* (the Royal Society of Chemistry), initiated by the late John D. Faulkner in 1984, and continued by Blunt and Munro and their colleagues from 2003. These reviews draw attention to a large number of other contributions devoted to more specific aspects and overviews of marine natural product chemistry. Sponges have been amongst the most studied of marine organisms and furnish a large proportion of marine natural products. Members of the genus *Plakortis* (phylum *Porifera*, class *Demosporgiae*, order *Homosclerophorida*, family *Plakinidae*) are particularly fascinating with respect to the variety of unusual metabolites they generate. Many *Plakortis* derived compounds exhibit anti-bacterial, anti-fungal, anti-tumor and other important pharmacological activities. Our interest in certain metabolites of *Plakortis* species encouraged us to summarize the important chemistry of these sponges in this review, with the hope this compilation will constitute a useful platform and aid further progress in this area.

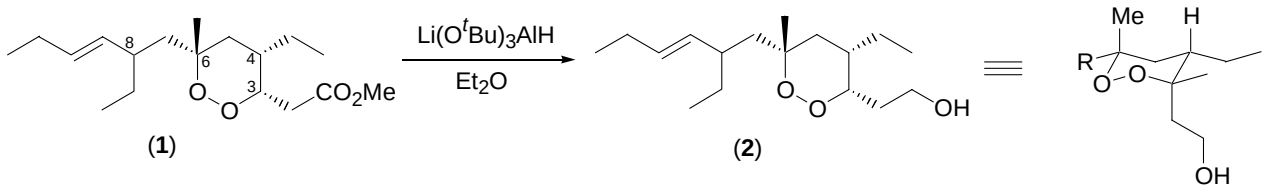
The review is structured on the basis of the metabolites from individual *Plakortis* species, followed by a discussion of synthetic contributions. The majority of investigations concern two species – *P.*

This paper is dedicated to Dr. Pierre Potier on the occasion of his 70th birthday.

*halichondrioides* and *P. simplex* – with limited studies on other species, some of which are taxonomically unclear, or undescribed. Data for these are included, if it is considered chemically relevant. With respect to metabolites described in this review, the stereochemistry represented in different reports, may vary. However, when an evidence-based stereochemical conclusion is arrived at, this is presented. A similar approach has been adopted with respect to varying names for the same compounds.

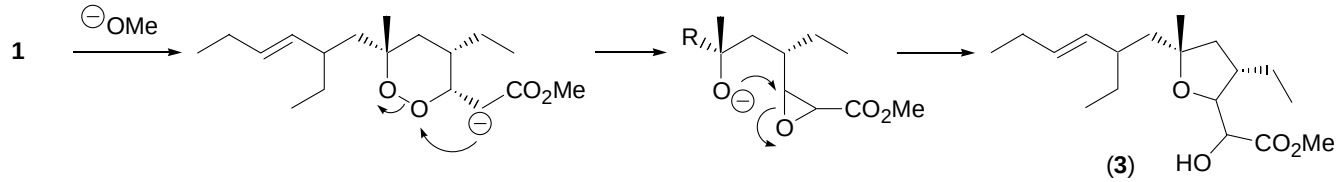
## METABOLITES FROM *PLAKORTIS HALICHONDRIOIDES*

In 1978 Faulkner examined the crude ethanol extract of this sponge, collected near Panama.[1] This extract inhibited growth of *E. coli* and *Staphylococcus aureus*, and the anti-microbial activity was connected with the predominant component of the metabolite mixture, which was named plakortin (**1**). A combination of spectroscopy and chemical degradation led to an unprecedented cyclic peroxide structure (**1**) for plakortin. Reduction with tri-*tert*-butoxyaluminum hydride furnished primary alcohol (**2**), and analysis of LIS (Lanthanide Induced Shift) data [Eu(fod)$_3$], led to the relative stereochemistry shown in Scheme 1, but that in the side chain (at C-8) was indeterminate.



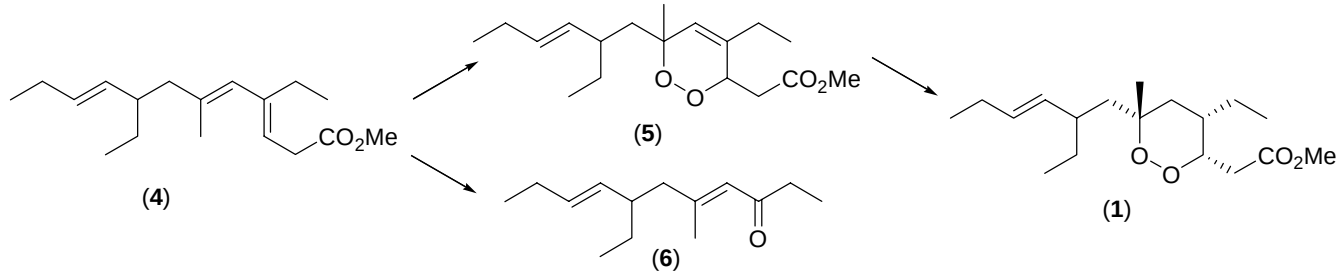

Scheme 1

Exposure of **1** to methoxide induced formation of a tetrahydrofuran (**3**) having an α-hydroxy ester side chain, postulated to result from initial cleavage of the peroxy group, as shown below.




Scheme 2

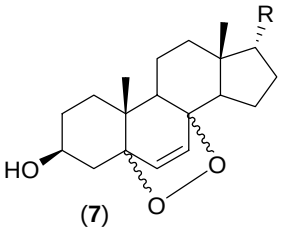
A ketone (**6**) occurred as a minor metabolite, and it and plakortin (**1**) can be considered to arise from a common 1,3-diene precursor (**4**).



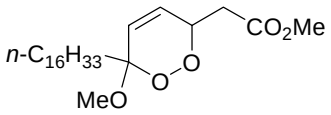

Scheme 3

Steroidal cyclic peroxides, e.g. (**7**), apparently resulting from autoxidation, and similar cases, e.g. (**8**), are

known. The fact that plakortin (**1**) is optically active, $[\alpha]_D^{20}$ +189 º ($CHCl_3$), was taken to mean that enzyme-mediation was involved.




(**7**)

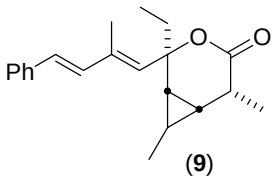



(**8**)

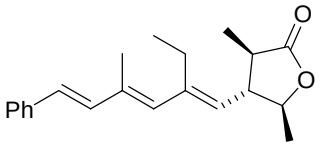
Optically active;
enzyme mediated oxygenation

Subsequently, Faulkner and co-workers examined several sponges from Belize,[2] which resembled the original sample of *P. halichondrioides*, but were distinguishable from it. Although some samples were *P. halichondrioides* and contained peroxides other than plakortin (**1**), another "abnormal" sample of *P. halichondrioides* lacked peroxides, but contained a series of substituted benzenes. A major component was the cyclopropane-containing δ-lactone (**9**), as well as the γ-lactone (**10**), and the minor γ-lactone (**11**), all with the indicated relative stereochemistry, based on LIS analyses.

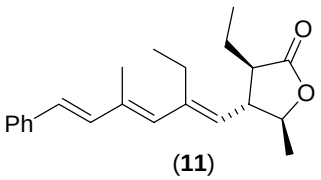
An α-hydroxy ester (**12**), tetraenes (**13**) and (**14**), and ketone (**15**) were minor metabolites.



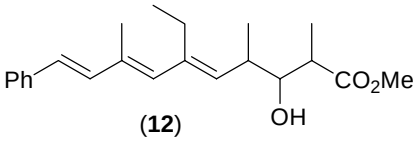

(**9**)



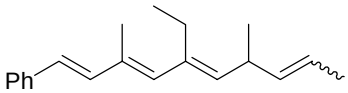

(**10**)



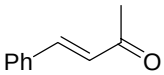

(**11**)




(**12**)

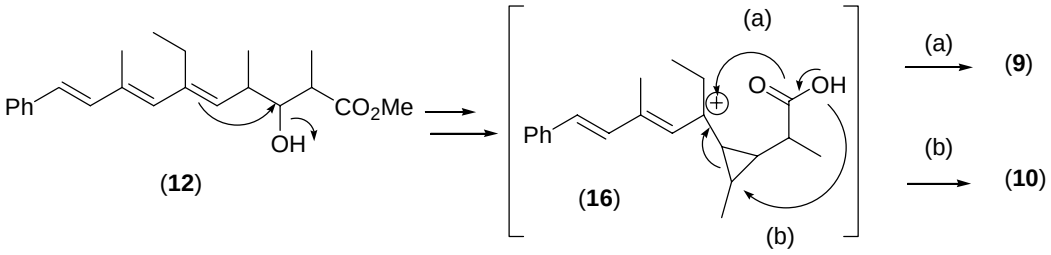



(**13**) and (**14**)




(**15**)

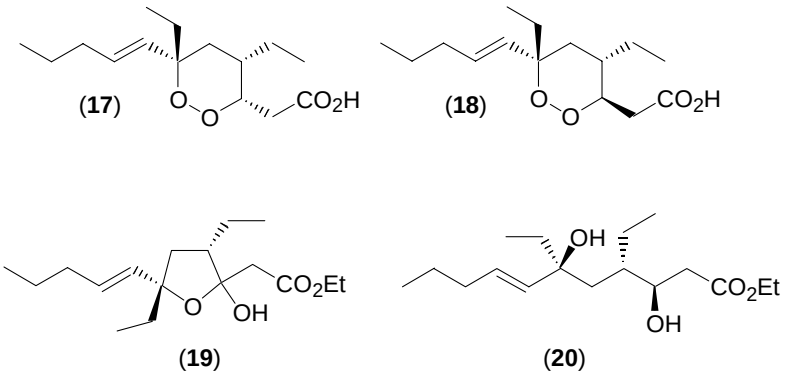
The seeming lack of relation of compounds (**9-15)** to plakortin (**1**) or ketone (**6**) encouraged re-confirmation of the taxonomy of the sponge, and other collections did incorporate other peroxides. However, hydroxy ester (**12**), itself notionally derivable by a mixed polyketide pathway, may be a key intermediate for lactones (**9**) and (**10**), with varying quenching of the cyclopropylcarbinyl cation (**16**).



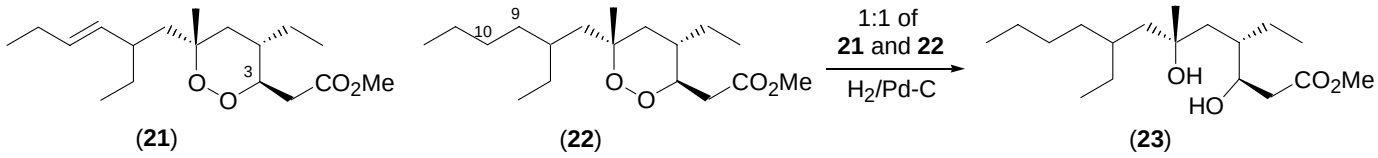

Scheme 4

Further studies by Faulkner, reported in 1980,[3] described additional metabolites from the Belize collection. Of the six samples, one was identified as *Chondrosia collectrix*, and contained the peroxides

(**17**) and (**18**),[4] whereas an ethanol-stored portion of the same material contained mainly the hemiketal (**19**) and diol (**20**). The other five samples of sponges were either *P. halichondrioides* or *Plakortis* sp., and the metabolites of two samples are described above.[2]

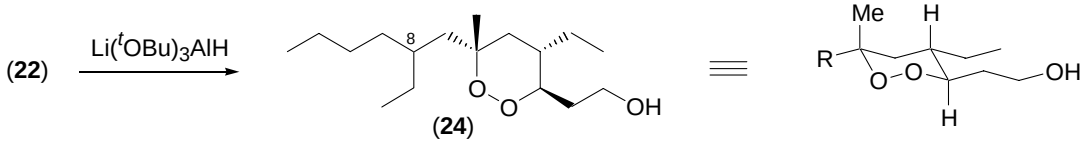



The remaining three samples provided additional examples of peroxides, related to plakortin (**1**), as well as esters, lactones and hydrocarbons, some of which were unstable. 3-*Epi*-plakortin (**21**) and 9,10-dihydro-3-*epi*-plakortin (**22**) were characterized by NMR spectroscopy, chemical correlations, and degradations. For example, a 1:1 mixture of **21** and **22** yielded a single diol (**23**) upon hydrogenation. A LIS study led to the relative stereochemistry.




Scheme 5

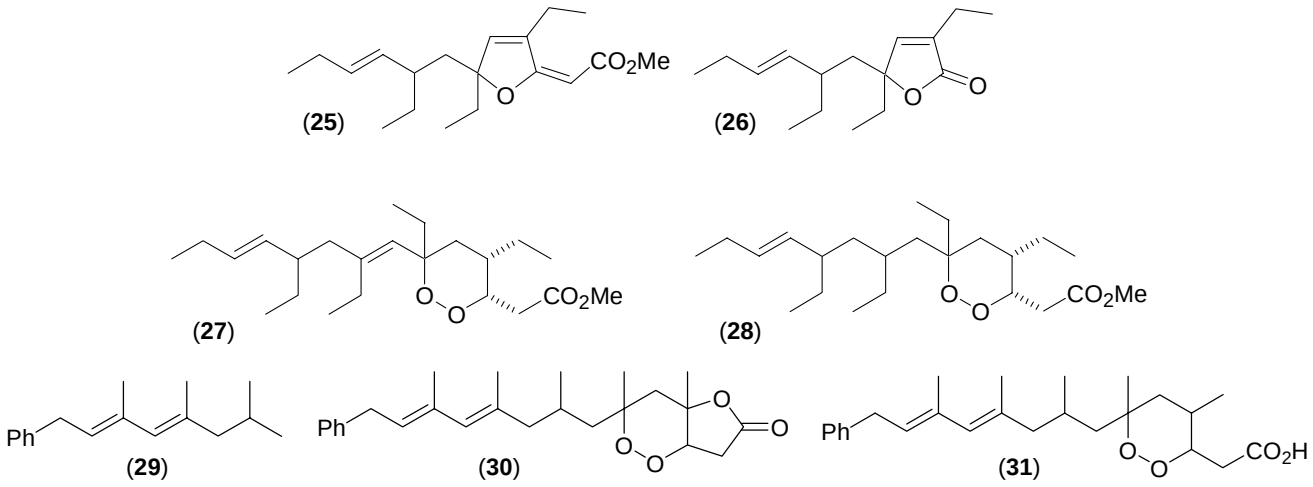
Reduction of the ester group of **22** with lithium tri-*tert*-butoxyaluminum hydride provided the peroxy alcohol (**24**), whose ring stereochemistry was confirmed by an LIS study, with C-8 stereochemistry again undetermined.




Scheme 6

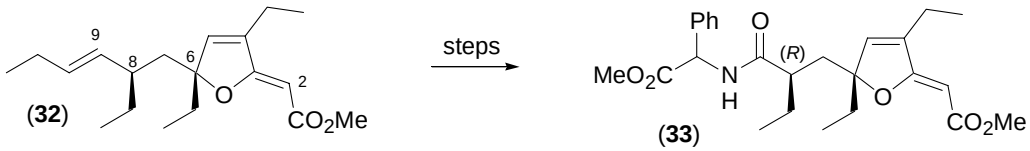
Another sample of *P. halichondrioides* was stored in ethanol and part was frozen. From the former, the ester (**25**) was characterized, whereas the frozen sample also provided **25**, as well as the γ-lactone (**26**) and peroxy esters (**27**) and (**28**). Free acids related to **27** also occurred.

The first sample contained unstable metabolites, but the evidence pointed to the existence of hydrocarbon (**29**), lactone (**30**) and an acid (**31**), all of undetermined relative stereochemistry.




These studies of Faulkner[3] resulted in the identification of unusual peroxides and related metabolites, probably tracing to hydroxy acids or 1,3-diene units formed by the mixed polyketide route, and accounting for substituent variations in 1,3-arrangements. The timing and mechanisms of oxygenation were not clear from these initial studies of the genus.

Progress in the determination of the absolute stereochemistry of *P. halichondrioides* metabolites was reported by Schmidt and Faulkner in 1996.[5] The methyl ester (**32**) was shown to possess (6*R*,8*R*) stereochemistry by chemical modification and use of Mosher's method.

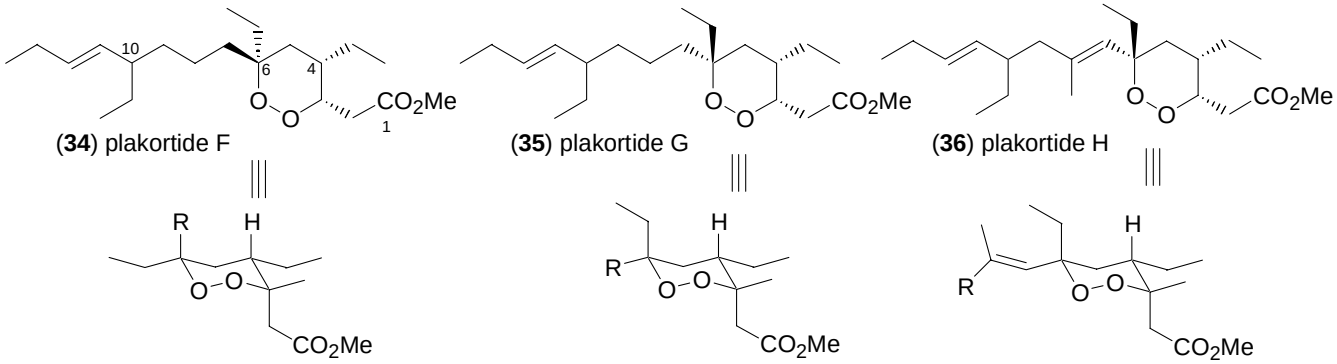



Scheme 7

With the (8*R*)-configuration determined, it was possible to relate this to the C6-configuration, shown to be (*R*) also. Thus **25** has (2*Z*,6*R*,8*R*,9*E*) stereochemistry, as portrayed in **32**.

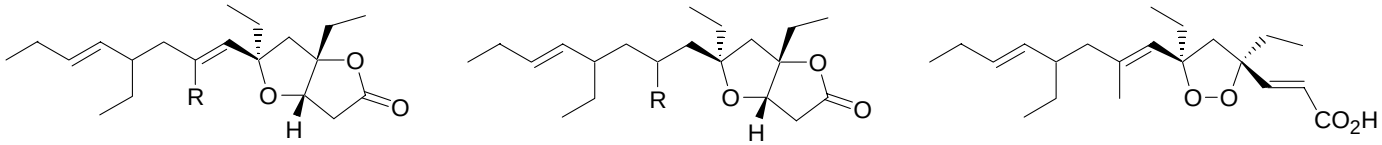
A high throughput screen with over 2400 marine and plant extracts by Patil and co-workers at SmithKline Beecham Pharmaceuticals,[6] revealed that the ethyl acetate extract of *P. halichondrioides* Wilson (*Plakinidae*) exhibited enhancement of SR-$Ca^{2+}$-ATPase activity and of SR-$Ca^{2+}$ uptake. Processing of this extract provided two known and eight novel compounds, with the former being 3-*epi*-plakortin (**21**) and the α,β-unsaturated ester (**32**). A series of closely related cyclic peroxides, called plakortides F-H (**34**)-(**36**) were characterized by extensive NMR spectroscopic methods and the relative stereochemistry by NOE measurements. In no case, was the relative stereochemistry at C-10 able to be determined.

The plakortides below (and 3-*epi*-plakortin (**21**)) significantly promoted $Ca^{2+}$ uptake by the SR, with 3-*epi*-plakortin reaching a higher level, but all in the relatively high 10-100 μM range. Other comparisons indicate that the size of the hydrophilic side chain may be a determinant of activity.[6]



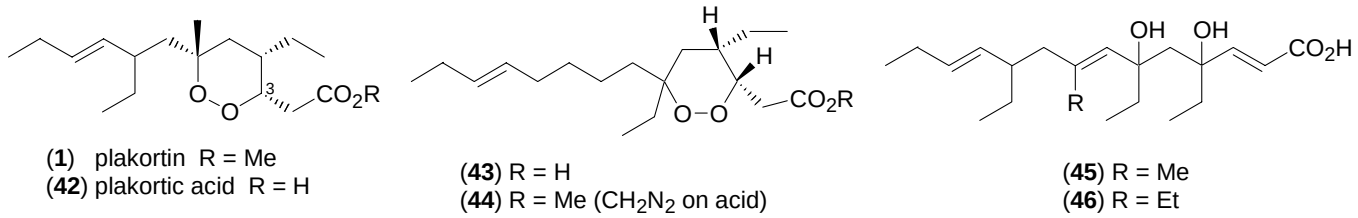

Scheme 8

A series of four bicyclic lactones – the plakortones A, B, C, and D – were also characterized in this study.[7] Their structures (below) were assigned by detailed spectral analyses, with the relative stereochemistry following from NOE studies.

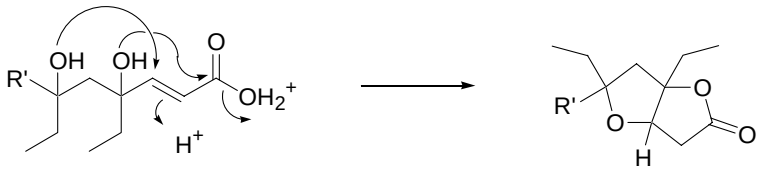



These novel lactones were examined for oxalate-supported $Ca^{2+}$ uptake using isolated cardiac SR-vesicles, and provided the basis for determining the effect of the lactones on SR-$Ca^{2+}$-pumping ATPase. All four plakortones significantly enhanced initial uptake rates, and plakortone D (**40**) exhibited the highest potency, active at micromolar concentrations. The activity of **40** encouraged its total synthesis, which confirmed its structure and absolute stereochemistry (see later). In the same report, an unusual peroxy acid, named plakortide E (**41**), was also described. This compound bore some spectral resemblance to the plakortones A-D, but incorporated a 1,2-dioxolane moiety, and an α,β-unsaturated acid.

A further study of *P. halichondrioides* from Jamaica furnished three new cytotoxic compounds,[8] with one acid being a likely precursor of the plakortones. Known compounds were a furano-α,β-unsaturated methyl ester (**25**), plakortin (**1**), 3-*epi*-plakortin (**21**) and plakortic acid (**42**). New compounds (**43-46**) are shown below.

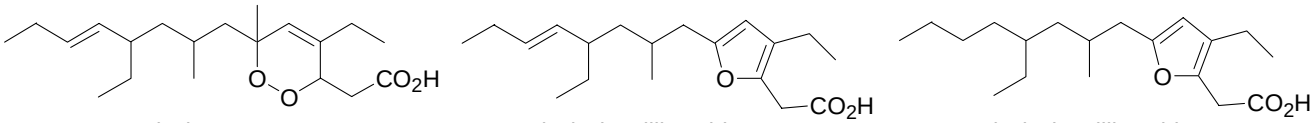



The open chain dihydroxytrienic acids (**45-46**) are notional, and perhaps, actual precursors of the plakortone series, derivable as shown below.



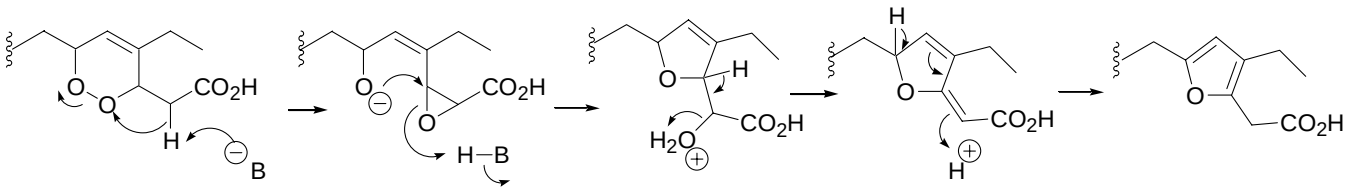
Scheme 9

Some Dominican sourced samples of *P. halichondrioides* (Glanvillia) and *Plakinastrella onkodes* (Capucin) have been investigated following the exhibition of *in vitro* cytotoxicity in crude extracts.[9] From the former sponge, the known cytotoxic peroxide (**47**), and two new, inactive, furans, – the glanvillic acids (**48**) and (**49**) – were characterized.



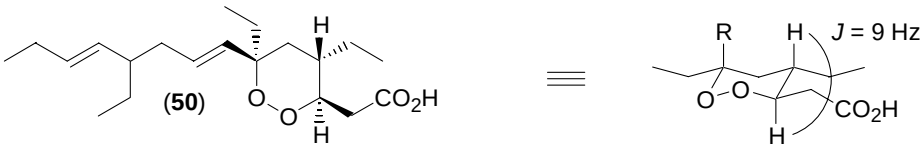
(**47**) (**48**) glanvillic acid A (**49**) glanvillic acid B

The cyclic peroxide (**47**) showed *in vitro* cytotoxicity, but no *in vivo* activity against murine leukemia P388. The glanvillic acids (**48**) and (**49**) were similarly inactive *in vitro*. The unusual C3-C6 furan moiety may be derived from a cyclic peroxide, as shown below.



Scheme 10

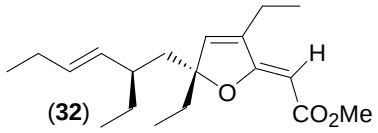
A search for compounds with anti-fungal properties led to examination of sponges in the family *Plakinidae*,[10] and ethanol extracts of *P. halichondrioides* and *Plakinastrella onkodes* inhibited the growth of the pathogens *Candida albicans* and *Aspergillus fumigatus*. A sample identified as *P. halichondrioides*, from Jamaica, provided the known metabolites, plakortides E, F, G and H, the trienoic acid (**45**), and the new 1,2-dioxane acid (**50**), with the relative stereochemistry below.



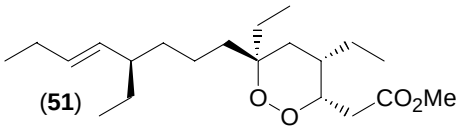
Scheme 11

Puerto Rican specimens of *P. halichondrioides* have also been examined and furnished known esters (**32**)[3] and (**51**)[6,7,8] along with two new plakortides O and P.[11] The relative stereochemistry of **53** was reported previously for other *Plakortis endo*-peroxides.[42,60] The absolute stereochemistry was deduced from NMR spectral analyses of various Mosher derivatives, to be (3*S*,4*S*,6*R*,10*R*), such configuration applying also to

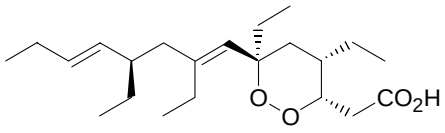
plakortide F (**34**). (The *endo*-peroxide (**50**),[10] is epimeric at C-3, but otherwise probably shares the stereochemistry assigned to plakortide O.) Plakortide P is likely to possesses the (3*S*,4*S*,6*R*,10*R*) configuration. Both new plakortides, O and P, exhibited potent cytotoxic activity against several cancer cell lines, but were not selective.



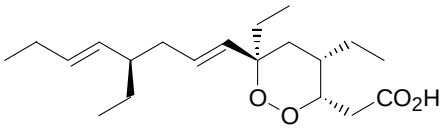

(**32**)




(**51**)




(**52**) plakortide P




(**53**) plakortide O

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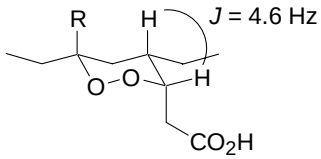
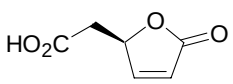
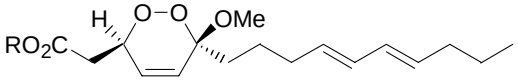



## METABOLITES FROM *PLAKORTIS SIMPLEX*

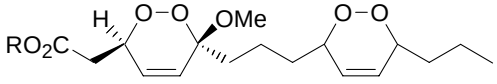
The first report describing metabolites from *P. simplex*, appeared in 1993.[12] A South African collection of *Plakortis* aff. *simplex* yielded a substituted butenolide (**54**), together with four new peroxy ketal compounds (**55**)-(**58**), these being the free acids and their methyl esters. All three free acids were very unstable, and the relationship between acid and ester was demonstrated by methylation with diazomethane. Arrival at these structures was assisted by the spectral data for methyl 5-butenolidyl acetate, xestins A and B (reported by Crews and co-workers from *Xestospongia* sp.),[13] and for chondrillin (described by Wells, from *Chondrilla* sp.).[57]



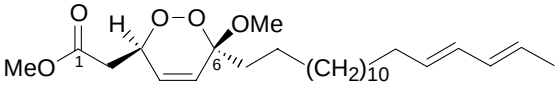

(**54**)



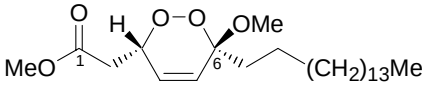

(**55**) R = H (**56**) R = Me




(**57**) R = H (**58**) R = Me




(**59**) xestin A
(**60**) xestin B (epimeric at C-6)

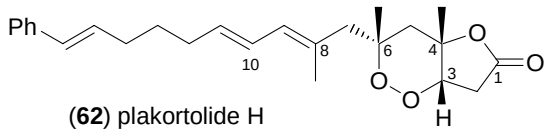



(**61**) chondrillin

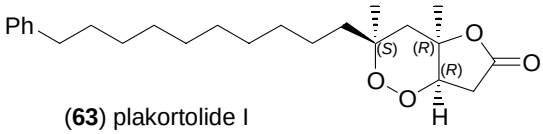
These comparisons indicated also that **55-58** had the same stereochemistry as xestin A about the 1,2-dioxene ring. The very unusual presence, perhaps the only one, of a di-dioxene system in **57** and **58** is to be noted, but the stereochemistry of the second heterocyclic ring was unclear. Both new methyl esters were cytotoxic to P388 murine leukemia cells ($IC_{50}$ < 0.1 μg/mL), but the free acids, expected to be more active, were too unstable for evaluation.

Further studies by the same group of a Madagascan collection of *P. simplex* have recently been reported.[14]

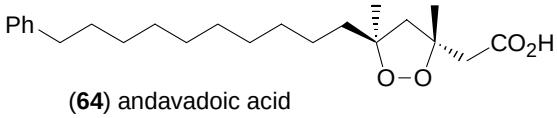
The crude extract was cytotoxic to a series of human tumor cells, and processing yielded two new 1,2-dioxane peroxylactones (plakortolides H and I) and a new 1,2-dioxolane called andavadoic acid.



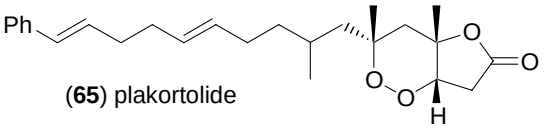

(**62**) plakortolide H




(**63**) plakortolide I




(**64**) andavadoic acid



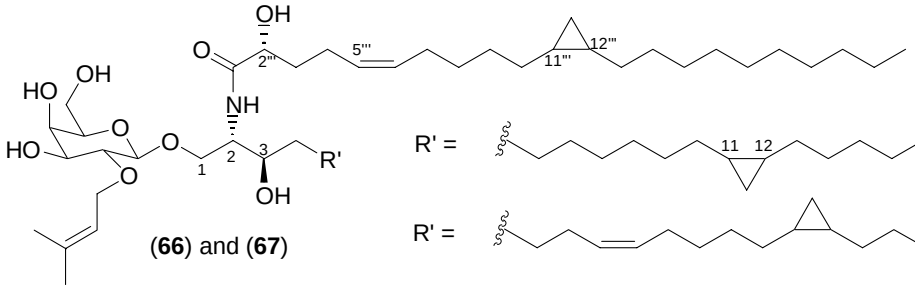

(**65**) plakortolide

Plakortolide H spectrally resembled plakortolide, previously reported by Davidson,[15] and was concluded to have the same relative stereochemistry and thus was the $\Delta^{8,10}$ diene analogue. Plakortolide I exhibited $[\alpha]_D$ +8 º, whereas a stereoisomer reported by Faulkner,[16] with (3*S*,4*S*,6*R*) stereochemistry, 6-epiplakortolide E (**376**) (see below),[101] had $[\alpha]_D$ –8 º. Plakortolide I is, on this basis, the (3*R*,4*R*,6*S*) isomer.

NMR spectral comparisons between andavadoic acid, the *epi*-plakinic acids[17] and a 1,2-dioxolane peroxy acid from *Plakinastrella onkodes* (for this sponge, see later), confirmed[10] the *trans* ring stereochemistry. Andavadoic acid was responsible for the non-selective cytotoxicity of the extract, with $GI_{50}$ in the submicromolar range against 13 human tumor cell lines.

Italian groups have undertaken detailed studies of *P. simplex* in the last five or six years,[18] and in 1997, Fattorusso and his associates described two unusual glycosphingolipids (GSL's) from a sponge collected from Little San Salvador Island in 1992,[19] as part of a general search for new active GSL's from a different genera. Previously the sponge genus *Agelas* had furnished compounds with immunostimulatory capability.[20]

The new plakosides A and B were purified as their acetyl derivatives and then deacetylated to provide the plakosides which were characterized by spectroscopic and microdegradation methods.[19]




(**66**) and (**67**)



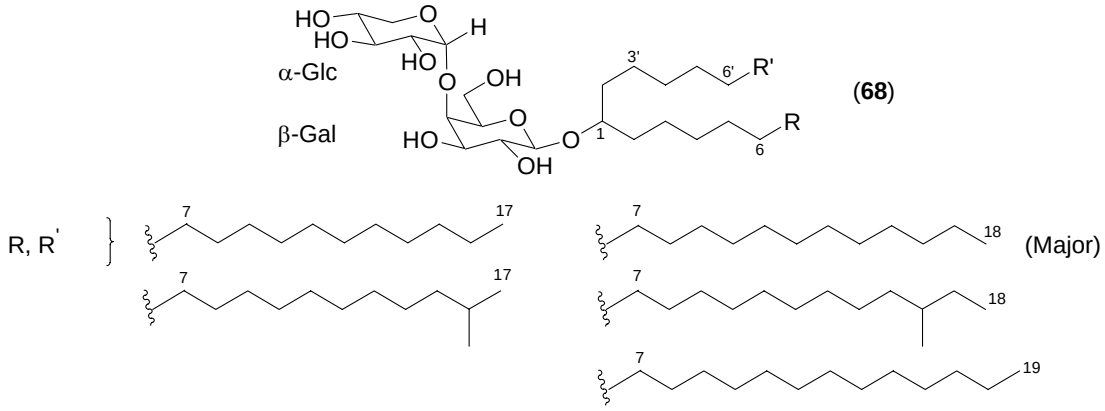

(**66**) plakoside A

(**67**) plakoside B

Thus plakoside A was determined to be (2*S*,3*R*,11*R**,12*S**)-1-*O*-[2-*O*-(3-methyl-2-butenyl)-β-D-galactopyranosyl]-2-[(1*R*,11*R**,12*S**)-1-hydroxy-11,12-methylene-5-docosenamido]-11,12-methylene-1,3-docosanediol. Glycosphingolipids with a prenylated sugar were previously unknown and the presence of the cyclopropane rings on the ceramide unit is also an unprecedented feature of these plakosides. Both

**66** and **67** demonstrate an immunosuppressive activity, which is attributable to the unusual 2'-*O*-dimethylallyl group, and overall shows the central importance of this 2'-hydroxyl group for immunological characteristics of GSL's in general. The very unusual structure and biological activity of the plakosides made the system a synthetic target[95,96,97] and will be discussed later.

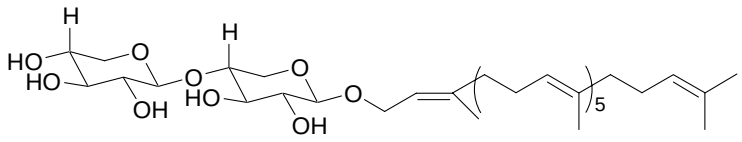
Further contributions by Fattorusso's group confirmed that marine sponges were a fertile source of unusual glycolipids. The simplexides (**68**) were identified as long-chain secondary alcohols glycosylated by a disaccharide unit, and constituted a new type of immunosuppressive glycolipids.[21]




(**68**)

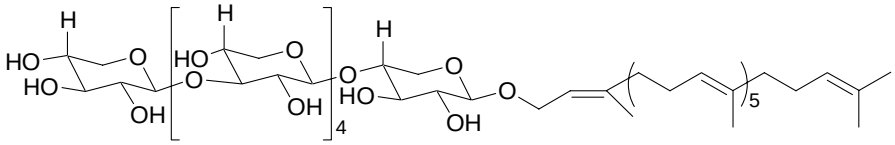
The simplexides are a complex mixture of homologues of differing lengths and branching in the alkyl chains, with the hydroxyl group located in the "middle" of the alkyl chain (34-37 carbons). The lipid portion of the simplexides was thought to result from a Claisen type of coupling of two fatty acid units. The simplexides showed pronounced inhibitory activity on the proliferation of murine immune-system T-cells stimulated with Concanavalin-A (ConA), and this resembled the activity of the plakosides from the same *P. simplex*, although the groups are quite dissimilar in the aglycone and the sugar moieties.

A cytotoxic glycolipid based on a linear C35 polyisoprenoid alcohol and a dixylosyl chain was isolated from *P. simplex* (Bahamas) in its pentaacetate form, using trideuteroacetic anhydride to ensure that the natural compound was not (originally) acetylated. The unusual structure was deduced from NMR spectral data and represents the first member of a new class of glycolipids.[22] Plakopolyprenoside exhibited an $IC_{50}$ of 4.5 μg/mL towards the J774 (murine) cell line.



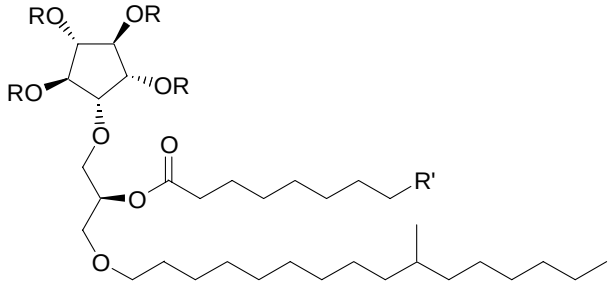
(**69**) plakopolyprenoside

This sponge furnished a further novel glycolipid, called plaxyloside (**70**).[23] This incorporated the same polyprenyl aglycone as in **69**, but a linear chain of six β-xylopyranoses as the carbohydrate unit. Microscale degradation and spectroscopic analyses of the peracetate led to the structure. Certain NMR characteristics of these oligosaccharides with repeating structures, were established.



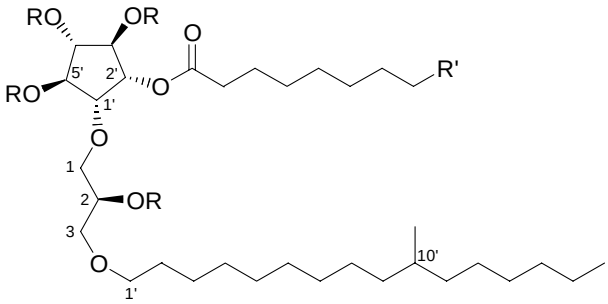
(**70**) plaxyloside

Crasserides represent another class of glycolipids and are very widely distributed in sponges including *Plakortis simplex* Schultze (family *Plakinidae*).[24] Further study of *P. simplex* from the Bahamas established that low levels of the isomeric isocrasserides were also present.



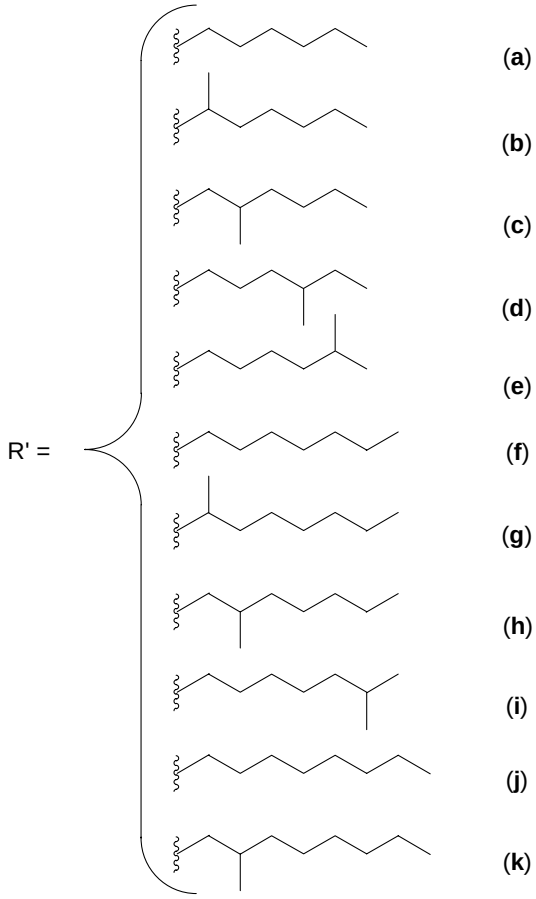
(**71**) R = H (**72**) R = Ac

crasserides



(**73**) R = H (**74**) R = Ac

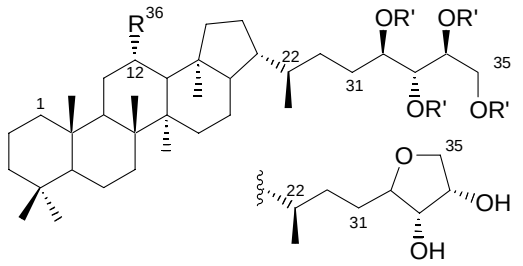
isocrasserides




A possible complication is that acetylation of the natural product to assist isolation and purification may induce acyl migration and ester interchange. However, isocrasserides (R = H) were isolated and spectral analyses confirmed the 2'-appendage of the acyl group. No ester interchange occurred during $SiO_2$ chromatography either, and this is strong evidence that isocrasserides and crasserides are authentic metabolites from *P. simplex.*

Fattorusso and co-workers have described the presence of bacteriohopanoids from *P. simplex*.[25] Such compounds are of mixed biosynthetic origin as they incorporate a hopane-based terpenoid which is linked *via* the 2-propyl group to a sugar derived five-carbon unit. These compounds act as membrane stabilizers in bacteria from which isolation occurred about 25 years ago, and a number of structural variants have

been characterized. *P. simplex* yielded large amounts of two bacteriohopanoids, the known bacteriohopanetetrol (**75**), and the new 12-methylbacteriohopanetetrol (**76**), possessing a methyl group at C-12, a feature not then known among the bacteriohopanoids, or hopane type terpenes. It was suggested that the unusual α-12-methylation might be carried out by the sponge, following acquisition of dietary or symbiotic bacteria.

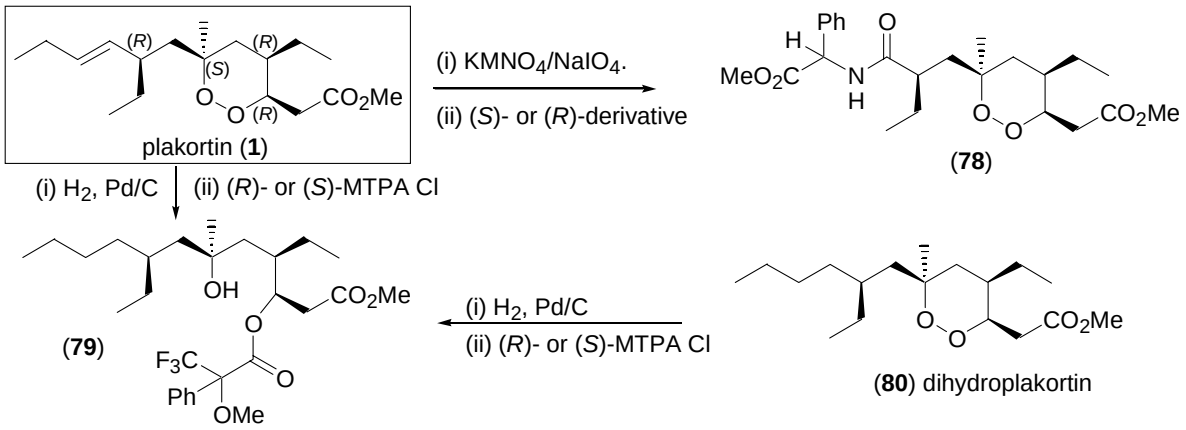



(**75**) R = H, R' = H (known)
(**76**) R = Me, R' = H (new)

(**77**) R = H

A more thorough examination of the lipophilic extract of the sponge *P. simplex* revealed large quantities of another new bacteriohopanoid, the 32,35-anhydro compound (**77**).[26] This new material was accommodated into the general biosynthetic scheme for the conversion of hopanes into bacteriohopanoids, and the large amounts of the compounds, as high as 50% in weight compared to sterols, reinforces a possible cellular structural role in *P. simplex*.

The Italian group, as part of further studies of *P. simplex* described the determination of the absolute stereochemistry of plakortin (**1**),[27] first reported by Faulkner in 1978,[1] from *P. halichondrioides*, but also quite abundant in *P. simplex*. Faulkner had established the relative stereochemistry of the peroxy ring, by an LIS study. Tactical degradation of plakortin (**1**), by both oxidation and reduction produced an acid, and a diol respectively, which were derivatized as shown below. Analysis of the Δδ (*S*-*R*) values led to assignment of (*R*)-configuration at C-8, and at C-3. With the known relative stereochemistry, the complete absolute stereochemistry was deduced, and shown below.

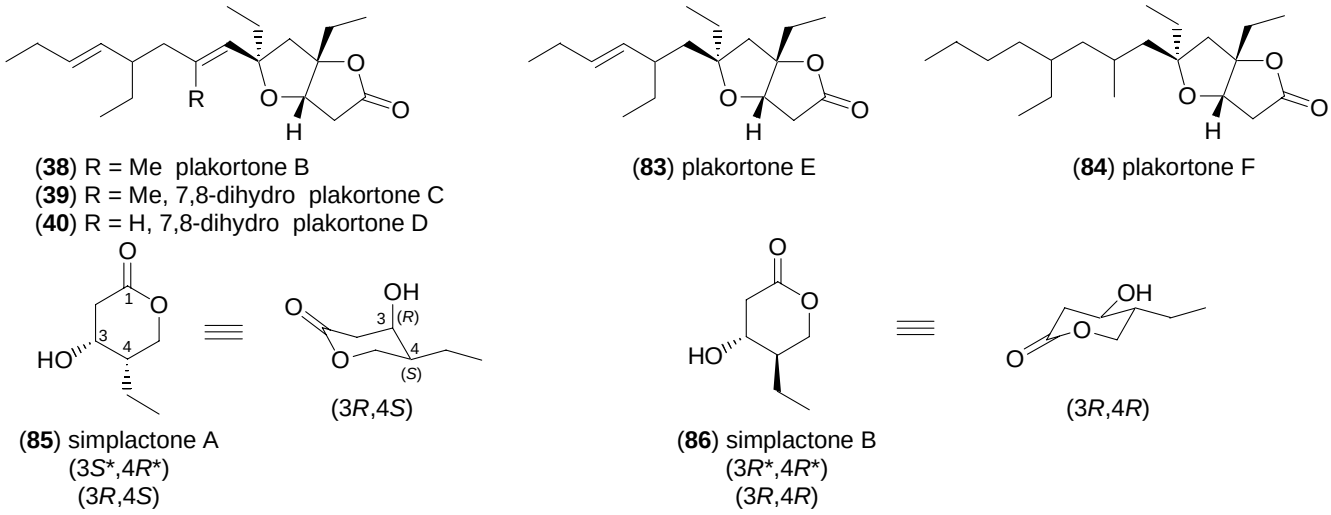



Scheme 12

Dihydroplakortin (**80**) was also isolated and correlated stereochemically, by reduction, with plakortin. Two further metabolites (**81**) and (**82**) were examined, and shown to possess the structures and absolute stereochemistry shown below, with that of the dihydro ester (**82**) being related to that of dihydroplakortin,

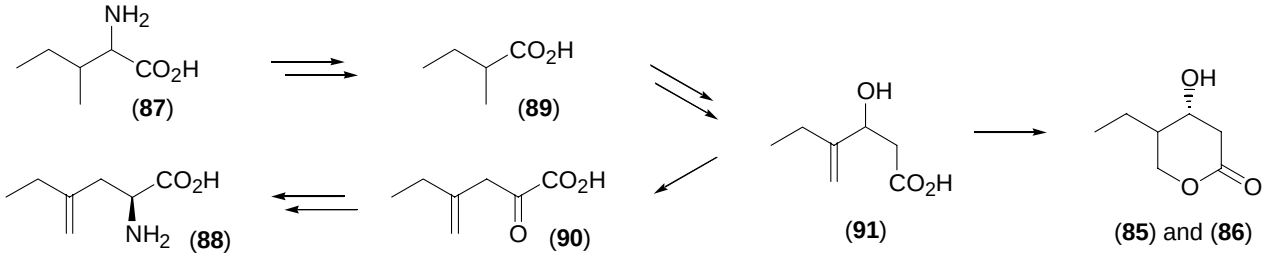
a likely precursor. Plakortin (**1**) was shown to be cytotoxic ($IC_{50}$ 7.0 μg/mL) whereas its dihydro derivative (**80**) was much less active ($IC_{50}$ >20 μg/mL).


The astonishing diversity of metabolites generated by *P. simplex* was further emphasized by the characterization of seven lactones including the known plakortones B, C, D, and new plakortones E and F and two new δ-lactones, simplactones A and B.[28]



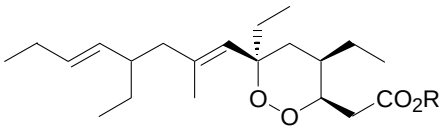

Scheme 13

(+)- And (–)-MTPA esters confirmed that simplactone A was (3*R*,4*S*), and simplactone B was (3*R*,4*R*). The simplactones exhibited mild cytotoxicity, but were inactive as antibiotic agents, and a larger alkyl chain in the δ-lactone system may be necessary for antibiotic activity. The unusual amino acid (**88**) probably originates from isoleucine (**87**), and this conversion may also afford an opportunity for simplactone formation as well.

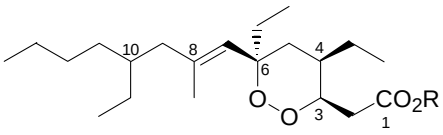



Scheme 14

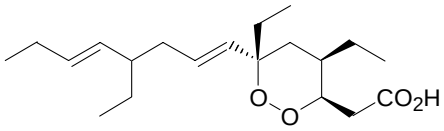
Further examination of *P. simplex* revealed the presence of diol analogues of plakortin or plakortide cycloperoxides, along with the known plakortide H[29] and new endoperoxides plakortides I and J.[30] The "ring opened" compounds, *seco*-plakortide H (**95**) and *seco*-plakortide I (**96**) were of considerable structural novelty.



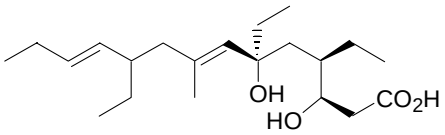

(**92**) R = H plakortide H



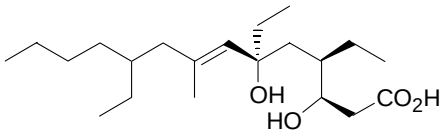

(**93**) R = H plakortide I




(**94**) plakortide J



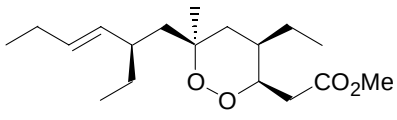

(**95**) *seco*-plakortide H



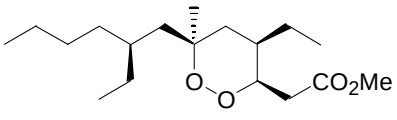

(**96**) *seco*-plakortide I

The absolute stereochemistry of these compounds was based on chemical correlation and analyses of Mosher derivatives, and within this group, the variable stereochemistry about the peroxide ring is to be noted. The compounds characterized exhibited noteworthy cytotoxicity against WEHI 164, a murine fibrosarcoma cell line. The cycloperoxide series were more active than the acyclic diol analogues.

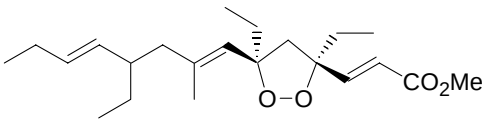
The quest for new antimalarials prompted evaluation of the *P. simplex* generated suite of *endo*-peroxides.[31] This was worthwhile because the *endo*-peroxide sesquiterpene lactone from *Artemisia annua* was known to be active against *Plasmodium falciparum*, with the *endo*-peroxide pharmacophore being critical for activity. The six-membered *endo*-peroxides, plakortin and dihydroplakortin, but not the five-membered cycloperoxide plakortide E, inhibited growth of *P. falciparum* parasites, with similar $IC_{50}$ values. Artemisin was, however, about fifty times more effective. These preliminary results should encourage further evaluation of sponge derived peroxides or their analogues. The compounds evaluated are shown below.[32]



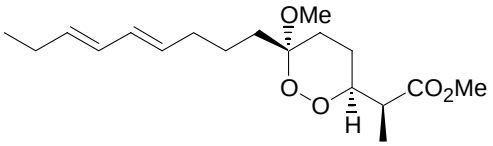

(**1**) plakortin



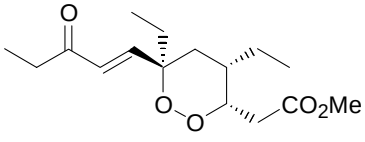

(**80**) dihydroplakortin



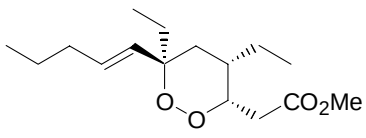

(**97**) plakortide E




(**98**) peroxyplakoric acid B3

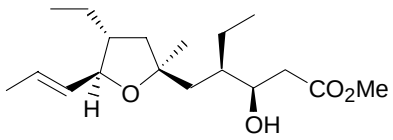


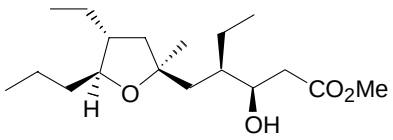

(**99**) plakortide M

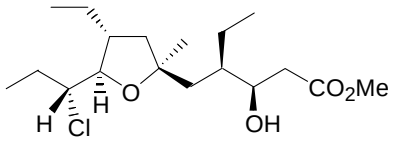


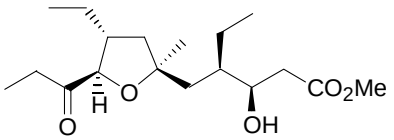

(**100**) plakortide K

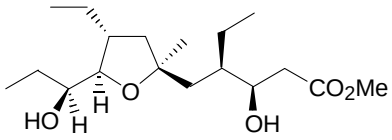
A new class of plakortin-type metabolites incorporating a trisubstituted tetrahydrofuran were isolated from *P. simplex*.[33] These are the plakortethers A-G, shown below, also a class of non-peroxide plakortin derivatives, and share the carbon backbone and absolute stereochemistry with plakortin (**1**).

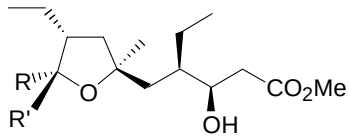

(**101**) plakortether A


(**102**) plakortether B

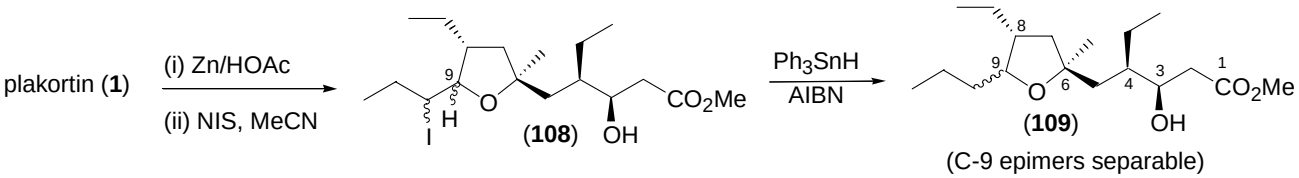

(**103**) plakortether C


(**104**) plakortether D


(**105**) plakortether E


(**106**) R = OMe, R' = H plakortether F
(**107**) R = H, R' = OMe plakortether G

The determination of absolute stereochemistry of plakortether B illustrates the general approach, and is based on the realization that the ether structures A and B possess the same skeleton as plakortin (**1**). Plakortin was transformed, as shown below, to plakortether B (and a separable C-9 epimer), one epimer being identical with plakortether B. This established that the configurations at C-3, C-4, C-6 and C-8 in plakortether B were the same as those in plakortin. The conversion of plakortether A into plakortether B confirmed their shared absolute stereochemistry.

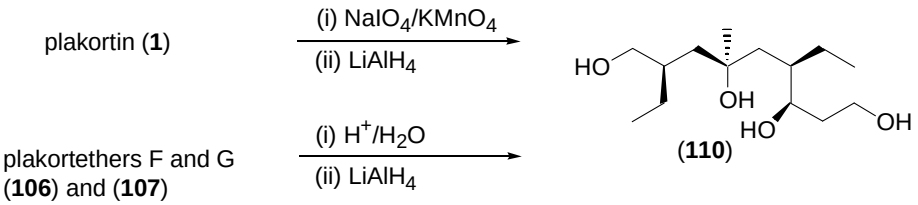



Scheme 15

The structure of plakortether C, which represents the first chlorinated metabolite from a *Plakortis* sponge is worthy of mention. The molecular formula was $C_{18}H_{33}O_4Cl$ (HRMS), and the NMR spectra indicated the same planar structure as for plakortether B, except for the chlorine atom, and interconversion was achieved by $Ph_3SnH$ reduction, showing that like descriptors (3*R*,4*R*,6*R*,8*R*,9*R*) applied for both compounds. The absolute configuration at C-10 (bearing chlorine) was deduced by application of *J*-based configuration analysis of Murata[34] to be (10*R*). Chemical correlations of the general types summarized above also permitted assignment of the absolute stereochemistry of plakortethers D and E as well.

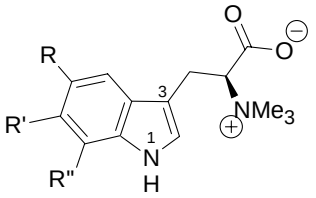
Plakortether F and G, shown to be epimeric at C-9, represent a fundamentally different group from the tetrahydrofurans of plakortethers A-E. Nevertheless, the absolute stereochemistry was correlated with that of plakortin by conversion to a tetrol (**110**) as shown below. Having then determined that F and G each share configurations at C-3, C-4, C-6 and C-8, the knowledge of the relative stereochemistry about the ketal system led to complete assignment of absolute stereochemistry to plakortethers F and G.

The suite of plakortethers was assessed for cytotoxic activity against two different cell lines representing murine fibrosarcoma and murine macrophage. All the compounds were inactive against the first cell line, whereas plakortethers A, B, D and E were selectively active against the second cell line. Among the $C_{18}$ plakortheters, only plakortether C (containing chlorine) was inactive.



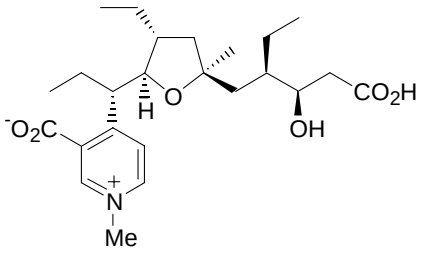

Scheme 16

The polar fractions from *P. simplex* provided three novel iodine-containing tryptophan betaines named plakohypaphorines A, B, and C (**111-113**),[35] which are structurally related to the known hypaphorine. These alkaloids represent the first examples of iodoindole derivatives from a natural source. Bromine and chlorine (of the halogens) are most frequently found in marine metabolites, whereas iodine-containing compounds are very rare. A brief discussion of this matter is presented in this report.[35] Subsequently, further plakohypaphorines D-F (**114-116**) were characterized from *P. simplex*,[36] and one (**115**) was the first natural triiodinated indole, and another (**116**) incorporated both chlorine and iodine in the indole nucleus. Evaluation of antihistamine activity of the plakohypaphorines A-F (**111-116**) was carried out, but only the diiodo derivatives produced a significant concentration-dependent reduction of histamine-induced contractions. Overall, the antihistamine activity seems to be related to the number and the nature of the halogen atoms on the aromatic ring.




(**111**) A, R = H, R' = H, R'' = I
(**112**) B, R = H, R' = I, R'' = I
(**113**) C, R = I, R' = H, R'' = I
(**114**) D, R = I, R' = I, R'' = H
(**115**) E, R = I, R' = I, R'' = I
(**116**) F, R = I, R' = H, R'' = Cl

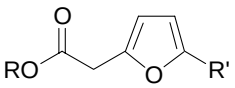
(**111**)-(**116**) plakohypaphorines A-F




(**117**) simplakidine A

*P. simplex* further revealed its enormous biosynthetic capability, with the isolation and characterization of simplakidine A,[37] an unusual 4-alkyl substituted pyridinium alkaloid (**117**), incorporating the THF-unit of the plakortethers, particularly plakortether B, shown above to possess (3*R*,4*R*,6*R*,8*R*,9*S*) stereochemistry. The portrayed stereochemistry of simplakidine A was based on a combination of methods and deduced to be (3*R*,4*R*,6*R*,8*R*,9*S*,10*S*). This metabolite has a $C_{17}$ polyketide moiety linked to a pyridinium ring. Pyridinium alkaloids are not unusual in sponges, but structural variations are limited. Simplakidine A showed low level cytotoxicity towards RAW 264-7 (murine macrophages) with 30% growth inhibition at 60 μg/mL, but less than that of plakortether B (50% at 9.5 μg/mL).

Chinese investigators in an examination of a Taiwanese *P. simplex* specimen, characterized several new furan derivatives, called plakorsins A-C (**118-120**), and a new fatty acid, plakortic acid (**121**),[38] in addition to some known metabolites.[39] The isolated compounds were assessed for cytotoxicity against *in vitro* human tumor cells, and plakorsin B exhibited strong activity against colon carcinoma.

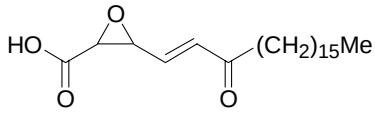



(**118**)-(**120**)
plakorsin A-C

(**118**) A, R = Me, R' = $CH_2(CH_2)_{14}Me$

(**119**) B, R = H, R' = $CH_2(CH_2)_{14}Me$

(**120**) C, R = Me, R' = $CH_2(CH_2)_6(CH_2CH_2CHO)Me$

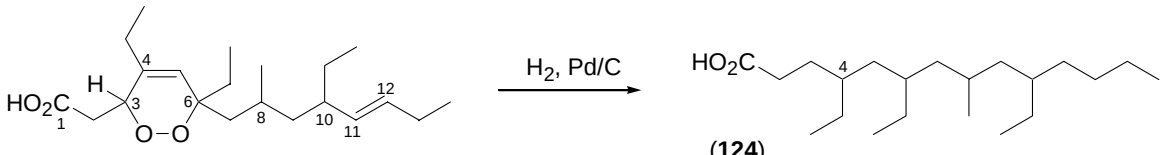



(**121**) plakortic acid

## METABOLITES FROM MISCELLANEOUS *PLAKORTIS* SPECIES

*Plakortis angulospiculatus*

A Venezuelan sourced sponge *P. angulospiculatus*, yielded two antifungal and cytotoxic cyclic-peroxy acids,[40] shown below, based on spectroscopic interpretations, and reduction to the saturated acid, as a mixture of C-4 epimers.



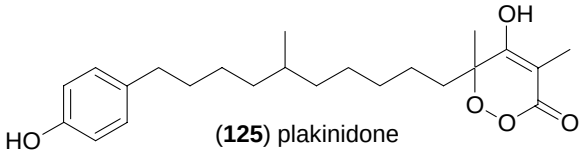

(**122**)
(**123**) 11,12-dihydro

(**124**)

Scheme 17

The relative stereochemistry was not able to be determined. Both peroxy compounds were active against *Candida albicans, Aspergillus nidulans,* and *Bacillus subtilis*, whereas the derived methyl esters ($CH_2N_2$) were inactive against all organisms. This is a general observation. Cytotoxicity against P-388 murine leukemia was also established.

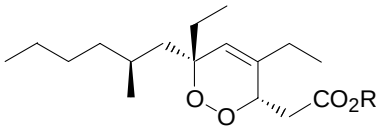
A further investigation by Faulkner was conducted on ostensibly the same sponge collected from the Tobago Cays.[41] An unusual perlactone, plakinidone, was characterized, and concluded to be the first natural product incorporating a six-membered perlactone ring. The crude methanol extract of the sponge inhibited the growth of the bacteria *Staphylococcus aureus* and *Bacillus subtilis* and *Herpes simplex I* virus, with activity concentrated in the dichloromethane fraction from the methanol extract. From this, plakinidone was isolated, but evaluation of this metabolite indicated major loss of activity during silica gel chromatography. The stereochemical aspects of the structure were not addressed. (The stereogenic centers are separated by five methylene groups.)



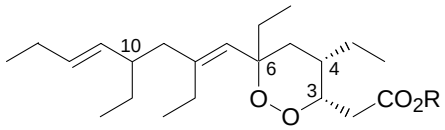

(**125**) plakinidone

The Palauan sponge *Plakortis* aff. *angulospiculatus* has been investigated as part of a program of drug discovery, with a particular focus on antileishmanial activity. (Leishmaniasis is a tropical disease that infects very large numbers in tropical Africa, Asia and South America, and is characterized by prolonged fever, and often spread by sand flies). Six new metabolites (**126-131**) were identified from two different

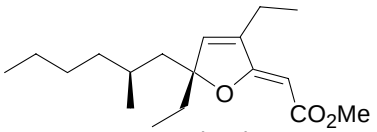
collections of this sponge and are shown below.[42]



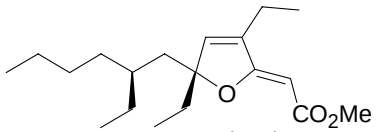

(**126**) R = H (**127**) R = Me



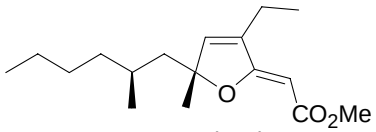

(**128**) R = H (**27**) R = Me




(**129**)

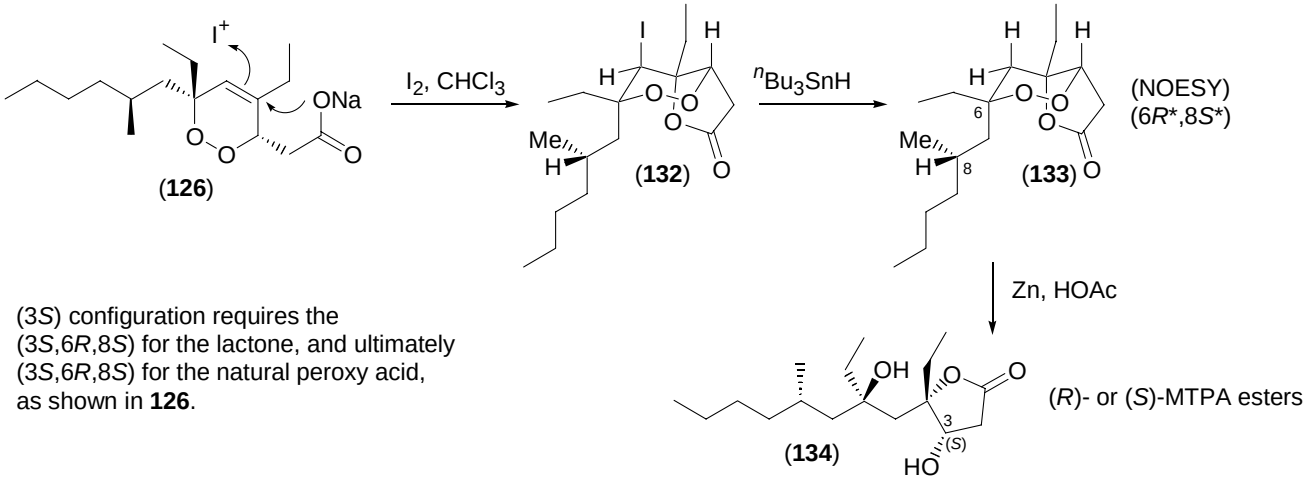



(**130**)




(**131**)

Elements of the relative stereochemistry of major peroxy acid (**126**) were determined by first converting it to the iodolactone, which proceeded with the expected *anti*-cyclization, as shown below.



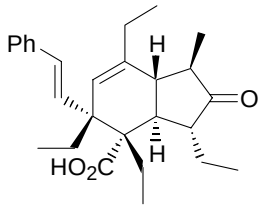

Scheme 18

The spectroscopic data for the second peroxy acid (**128**) indicated that it was the acid corresponding to a known methyl ester (**27**), generated by methylation of an acid mixture from *P. halichondrioides* from Belize.[3] Although NMR spectral comparisons confirmed the constitution and *cis*-3,4-relationship, further NMR spectral comparisons indicated the stereochemistry at C-6 and C-10 was indefinite.

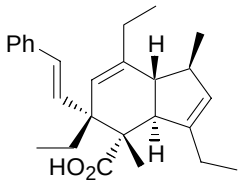
The major furan (**129**), from both samples, was deduced to have (2*Z*,6*R*,8*S*) stereochemistry, on the basis of comparisons with a homologue (8-ethyl vs. 8-methyl) from *P. halichondrioides,* and mechanistic argument for the base-induced ring contraction and elimination, for furan formation from the peroxy acid (**126**), with (3*S*,6*R*,8*S*) stereochemistry. The same (2*Z*,6*R*,8*S*) stereochemistry was deduced for the minor triethylfuran (**130**), and the minor 6,8-dimethylfuran (**131**) from the latter collection, was also assumed to have conforming stereochemistry. The major peroxy acids and furans were assessed for their effects on the proliferation of *Leishmania mexicana* promastigates, which was most sensitive to peroxy acid (**126**). Peroxy acid (**128**) and furans were less effective, and all less effective than ketoconozole.

Studies by Andersen's group of the Carribean sponge, *Plakortis angulospiculatus*, from Dominica, provided two novel polyketides,[43] the spiculoic acids A and B, with the former showing *in vitro*

cytotoxicity against human breast cancer MCF-7 cells, whereas spiculoic acid B, was inactive.

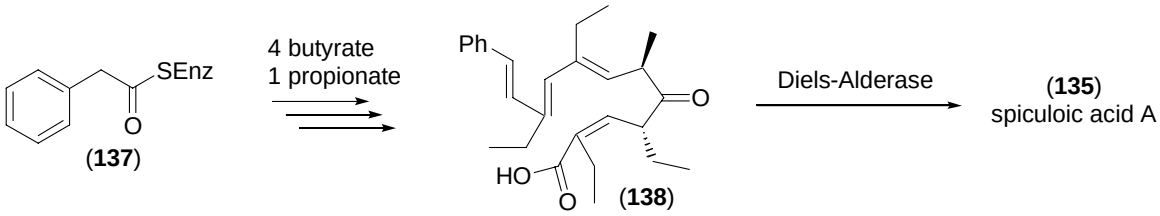



(**135**) spiculoic acid A




(**136**) spiculoic acid B

The similar relative stereochemistry of these metabolites was established by NOESY and scalar coupling constant data. The biosynthesis of spiculoic acid was concluded to involve a triene assembly from phenylacetic acid, butyrate and propionate units (to install four ethyl and one methyl groups), followed by a [4+2] cycloaddition perhaps catalyzed by a Diels-Alderase. Notice that the cycloaddition generates adjacent quaternary centers.

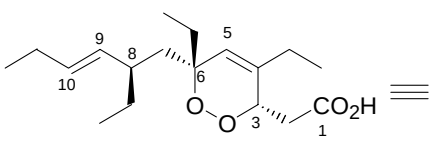



Scheme 19

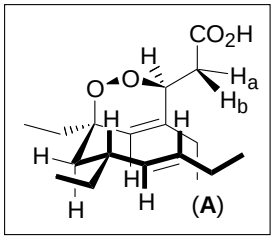
Many invertebrates, particularly *Plakortis sp.*, provide many examples of ethyl appendages, presumably resulting from butyrate incorporation.

*Plakortis lita*

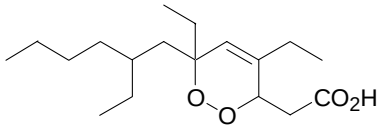
As part of a search for compounds that inhibited the cell division of fertilized sea urchin eggs, Uemura and co-workers described two cytotoxic endoperoxides – haterumadioxins A (**139**) and B (**140**) – from the Okinawan sponge *Plakortis lita* De Laubenfels.[44] The relative and absolute stereochemistry of haterumadioxin A was also deduced. Reduction ($LiAlH_4$), selective silyl protection of the primary alcohol and Mosher ester derivatization of the secondary alcohol led to assignment of (3*S*), and therefore (3*S*,6*R*,8*R*) for haterumadioxin , on the basis of a preferred conformation (**A**) based on *J* values and NOE correlations. These peroxides showed significant cytotoxicity against 38 human cancer cell lines.




(**139**) haterumadioxin A



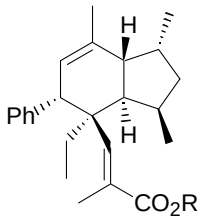

(**A**)



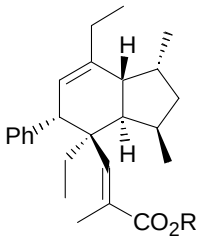

(**140**) haterumadioxin B

Another examination of this reddish brown encrusting sponge *P. lita*, was reported by Faulkner and co-workers,[45] as part of a search for compounds with anti-arthritic activity. In addition to the known

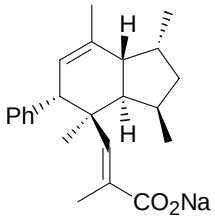
compound plakotenin (**141**),[67] its sodium salt, homo-plakotenin, its sodium salt and the salt of *nor*-plakotenin were also characterized. These compounds, plakotenin, its sodium salt and *homo*-plakotenin, significantly reduced proliferation of rheumatoid synovial fibrolasts.




(**141**) R = H plakotenin
(**142**) R = Na

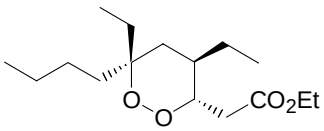



(**143**) R = H *homo*-plakotenin
(**144**) R = Na

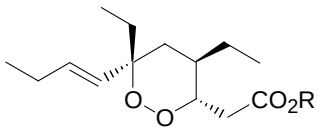



(**145**) *nor*-plakotenin

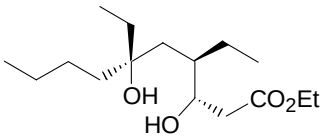
*P. lita* from Papua New Guinea was examined by Crews who reported three cyclic peroxides and three acyclic diol analogues.[46] The absolute stereochemistry was assigned by Mosher ester derivatization of a diol, and stereochemical regularity was assumed for the related series of compounds. As expected, the cyclic peroxides were generally cytotoxic, whereas the open chain diols were inactive. Other assays were also conducted.



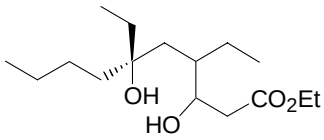

(**146**) ethyl plakortide Z



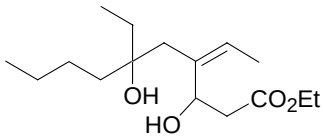

(**147**) R = Me methyl didehydroplakortide Z
(**148**) R = Et ethyl didehydroplakortide Z




(**149**) ethyl *seco*-plakortide Z



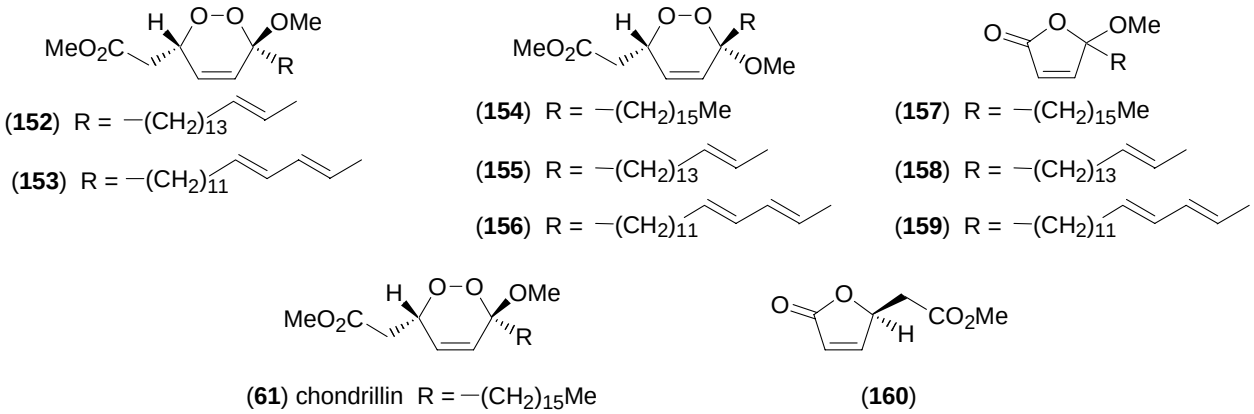

(**150**) *epi*-ethyl *seco*-plakortide Z



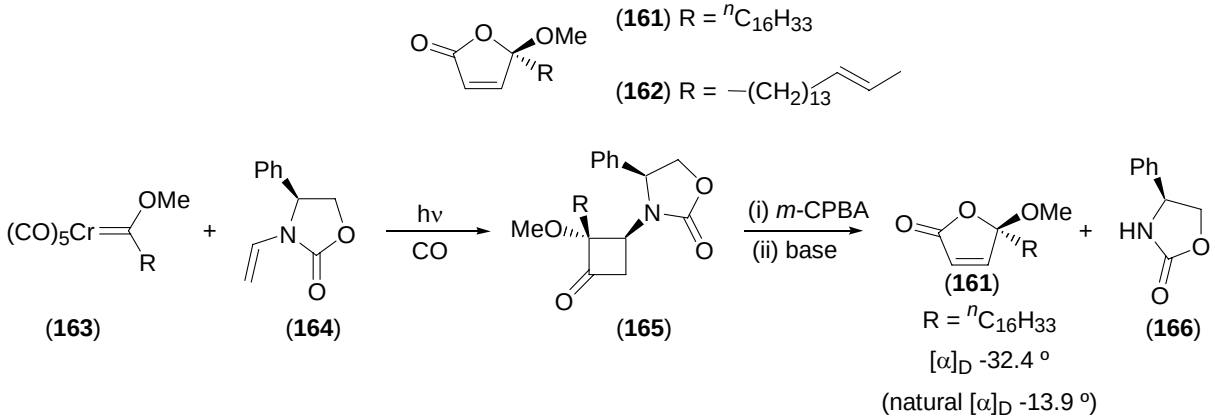

(**151**) ethyl didehydro-*seco*-plakortide Z

Further studies of *P. lita* by De Guzman and Schmitz emphasized the rich molecular diversity of metabolites from *Plakortis* species generally. The extracts of *P. lita*, collected at Truk (South West Pacific) provided five new cyclic peroxy esters and three new butenolides, shown below.[47] These compounds are related to the known and co-occurring chondrillin (**61**) and ester (**160**).

Neither chondrillin nor diene (**153**) showed significant cytotoxicity towards P388 leukaemia cells ($ED_{50}$> 10 μg/mL), a result in contrast with other reports for cyclic peroxy aliphatic esters, with $IC_{50}$ of 0.05-5 μg/mL.[13,48] This sample of *P. lita* (from Truk) yielded a suite of C-22 peroxy esters whereas *P. lita* from Okinawa provided chondrillin (C-22) as the major product. As a result the presence of peroxy aliphatic esters may not be useful in a taxonomic sense.

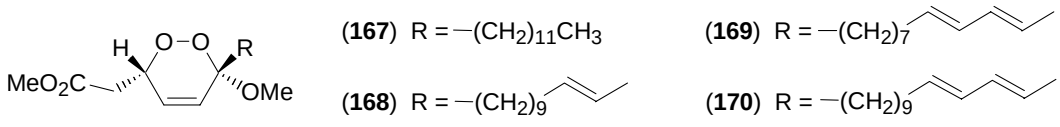



Synthesis of the butenolides below, from *P. lita*,[47] have been carried out, using chromium alkoxycarbene complexes as key intermediates,[49] and comparisons led to assignment of (*R*)-configuration to the natural compounds. Comparisons of optical rotations indicated the natural butenolides were inpure. Patent coverage of cyclic peroxide formulations, as present in *P. lita*, has been provided.[50]




Scheme 20

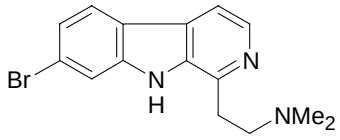
Other studies of *P. lita* by Higa and co-workers identified additional examples of cyclic peroxy esters with alkyl chains,[48] and indicate that a wide range of fatty acid based construction is possible. They are epimeric to chondrillin, and are about 50-100 times more active in *in vitro* anti-tumor assay against P388 cells, and imply considerable importance of the peroxy ring stereochemistry for activity. Again, a summary of the occurrence of these peroxy esters indicates they lack taxonomic significance.



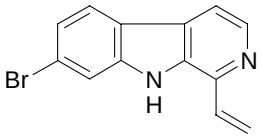

*Plakortis nigra*

The dark brown, deep water sponge *P. nigra*, collected in Palau, provided the first example of cytotoxic β-carbolines co-occurring with cytotoxic cyclic peroxides.[51] Generally speaking, cyclic peroxides are

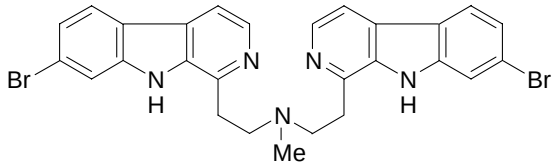
characteristic of the genera *Plakortis* and *Plakinastrella* whereas simple β-carbolines are associated with ascidians, and may have anti-viral activity. Difficult separation procedures eventually led to the isolation of the plakortamines A-D, two cyclic peroxides – the *epi*-plakinic acids G and H – and two γ-lactones. Most of these eight metabolites inhibited the HCT-116 human colon tumor cell line, with plakortamine B being the most active.



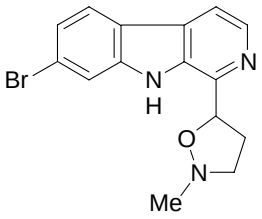
(**171**) plakortamine A



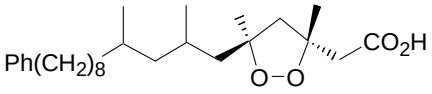
(**172**) plakortamine B



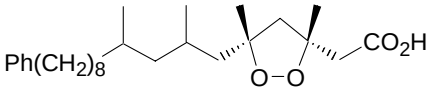
(**173**) plakortamine C



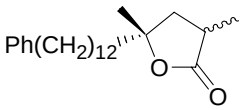
(**174**) plakortamine D



(**175**) *epi*-plakinic acid G

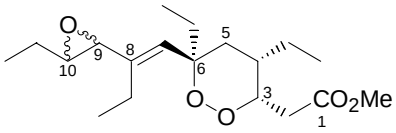


(**176**) *epi*-plakinic acid H



(**177**) and (**178**)

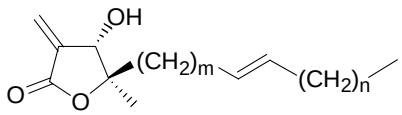
A very recent report describes two new cyclic peroxides from *P. nigra* (collected near Sulawesi, Indonesia) that inhibit cancer cell growth against the murine P388 lymphocytic leukemia cell line.[52] The relative configurations of plakorstatins 1 and 2 were determined, and these compounds are the first plakortides with an epoxy group in the side-chain. The stereochemical difference is associated with the epoxide configuration.



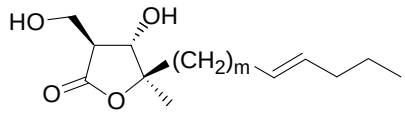
(**179**) plakorstatin 1: (9*S*,10*S*) or (9*R*,10*R*), $[\alpha]_D$ -177 º (MeOH)
(**180**) plakorstatin 2: (9*R*,10*R*) or (9*S*,10*S*), $[\alpha]_D$ -220 º (MeOH)

*Plakortis quasiamphiaster*

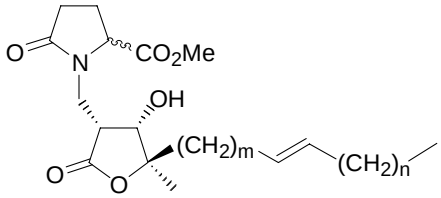
The South Pacific sponge *P. quasiamphiaster* has provided an unusual group of oxygenated, long-chain derivatives, called the amphiasterins, from the cyclotoxic extract.[53]



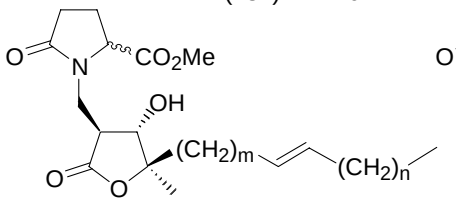
(**181**) m = 31, n = 9
(**182**) m = 31, n = 9 (satd)
(**183**) m = 35, n = 9
(**184**) m = 35, n = 9 (satd)



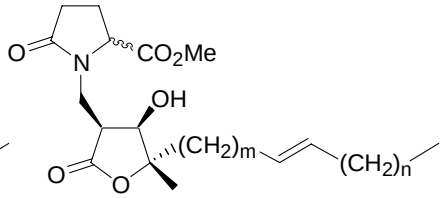
(**185**) m = 10
(**186**) m = 12
(**187**) m = 16
(**188**) m = 10 (satd)
(**189**) m = 12 (satd)



(**190**) m = 3, n = 9
(**191**) m = 3, n = 9 (satd)
(**192**) m = 5, n = 9
(**193**) m = 5, n = 9 (satd)



(**194**) m = 3, n = 9
(**195**) m = 5, n = 9
(**196**) m = 5, n = 9 (satd)



(**197**) m = 3, n = 9

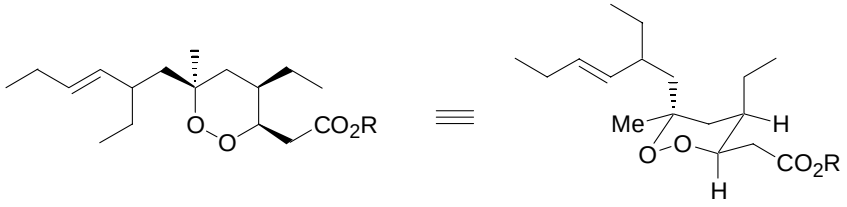
Some seventeen amphiasterins could be organized into five structural groups, differing in length or unsaturation of the alkyl side chain. These are shown above.

*Plakortis zygompha*

Faulkner has emphasized, that in contrast to the generally malodorous marine sponges, *P. zygompha* has a sweetish, fragrant distinctive odor, attributable to (Z)-7-methyl-4-octen-3-one (**198**), which could be obtained by trapping of emitted volatiles from a thawing sample.[54] The ethyl acetate soluble material afforded additional relatively simples ketones, alcohols and esters, some of which may have arisen during ethanol extraction, but possibly from a monoterpene. Other studies indicated that *P. zygompha* derived compounds caused a pronounced bleaching-necrotic effect on sponge tissue and coral.[55]

Another Caribbean sponge identified as *Plakortis zygompha* (de Lauberfels, 1934) provided the free acid (**42**) (named plakortic acid) corresponding to the methyl ester, characterized by Faulkner as plakortin (**1**).[1] Although plakortin (and the 3-epimer) co-occurred with plakortic acid, in this sponge, it was essentially inactive, in contrast to the report by Faulkner. The free acid (**42**), however, was a potent antibacterial and antifungal agent. The methyl esters of plakinic acids A and B (see below) were also inactive, whereas the free acids were active.[56]

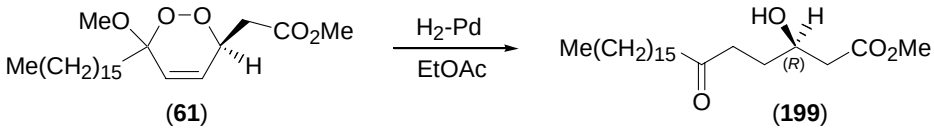
(**42**) R = H plakortic acid
(**1**) R = Me plakortin




Scheme 21

## METABOLITES FROM UNDESCRIBED *PLAKORTIS* SPECIES

Wells first reported a peroxy containing metabolite from a sponge in 1976.[57] A widely distributed member of the genus *Chondrilla* on the Great Barrier Reef (Queensland) furnished a compound, chondrillin, deduced to have structure (**61**), on the basis of spectroscopy and chemical degradation. The optical activity ($[\alpha]_D$ +144 º) was taken to indicate an enzyme-mediated process, and not an artifact from oxygen addition to a diene.



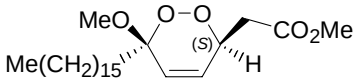

(**61**) (**199**)

Scheme 22

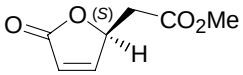
Reduction provided the secondary alcohol (**199)** which was considered to be (*R*)-configured (CD, ORD) and hence C-3 in **61** was (*S*).

Studies of the Okinawan sponge *Plakortis* sp. by Kobayashi are of relevance to the above, as a new cyclic peroxide called plakorin (**200**) was characterized,[58] and shown to be a potent activator of sarcoplasmic

reticulum (SR) $Ca^{2+}$-ATPase. The spectral data for plakorin resembled that for chondrillin (**61**) (above), but with a different optical rotation ($[\alpha]_D$ +44.3 º) and was concluded to be the C-6 epimer of chondrillin. A co-occurring but-3-enolide was shown to be (3*S*) configured, consistent with the (3*S*)-configuration for plakorin.

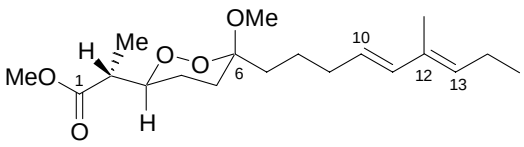


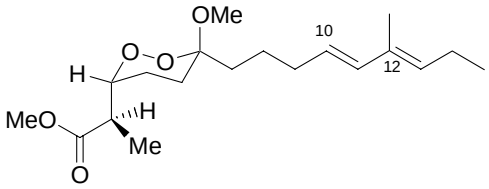

(**200**) plakorin




(**160**)

Further investigation by Kitagawa addressed the absolute stereochemistry of a group of anti-fungal peroxy ketal acids and esters previously isolated from this Okinawan *Plakortis* sp.[59] Reduction and analysis of shifts within derived (+)- and (–)-MTPA esters, provided the stereostructures shown below.

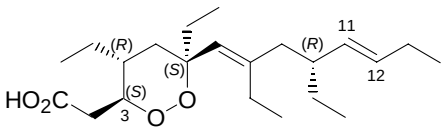


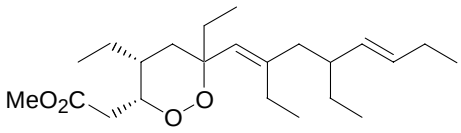




(**201**) peroxyplakoric acid $A_1$, methyl ester
(**202**) (12-13)-*Z*: peroxyplakoric acid $A_2$, methyl ester
(**203**) (12-13)-*E*, 12-desmethyl: peroxyplakoric acid $A_3$, methyl ester

(**204**) peroxyplakoric acid $B_1$, methyl ester
(**205**) 12-desmethyl: peroxyplakoric acid $B_2$, methyl ester

Additional peroxy compounds (**206-208**) from an Okinawan sponge, *Plakortis* sp. were reported by Kobayashi in 1998.[60] The absolute stereochemistry shown was based on analysis of MTPA esters, and additional analysis assigned (*R*)-configuration to C-10. Additionally, **207** and **208** were shown to share the same dioxane-ring absolute stereochemistry. This work provided considerable insight into the absolute configuration of these and similar plakortin-like endoperoxides. It was pointed out that **208** resembled **209** described by Faulkner[3] except for optical rotation, but the good agreement in partial $^1$H NMR spectral data may indicate the compounds are the same.

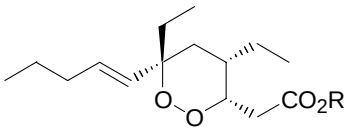


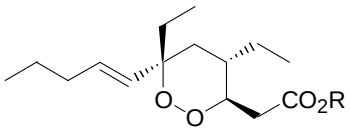

(**206**)
(**207**) is *3-epi*-11,12-dihydro (**203**) (3*R*,4*R*,6*S*)
(**208**) is 3-*epi* (**203**) (3*R*,4*R*,6*S*)

(**209**) ($[\alpha]_D$ -224 °)

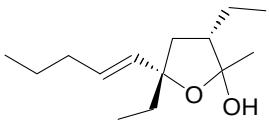
The Caribbean sponge *Chondrosia collectrix* is also a rich source of highly oxygenated metabolites,[4] but this report demonstrates the possibility of metabolite transformation and loss of initial activity on storage, and solvent derived artefacts. A dichloromethane extract of previously frozen lyophilized sponge provided a mixture of peroxy acids, their methyl esters and the hemiketal (**212**).




(**17**) R = H
(**210**) R = Me

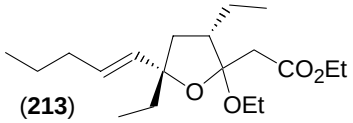



(**18**) R = H
(**211**) R = Me

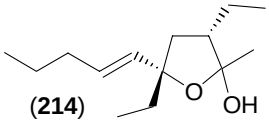



(**212**)

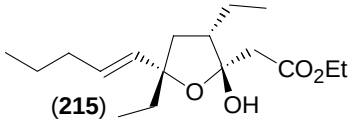
The peroxides were associated with moderate antibacterial activity. The ether-soluble portion of the ethanolic extract (3 months at 0 ˚C), in contrast, yielded no peroxides, but predominantly five-membered ring ketals and hemiketals, as shown below.



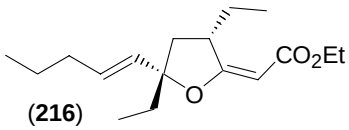

(**213**)



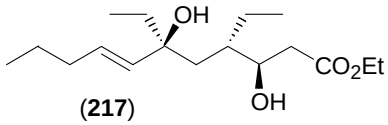

(**214**)




(**215**)



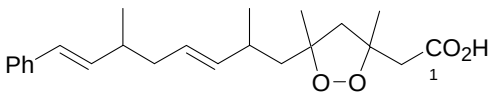

(**216**)



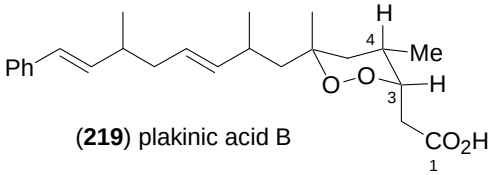

(**217**)

It is reasonable that the latter metabolites originate from the peroxy acids and esters, and as a consequence lose their antibacterial activity. Treatment of the hemiketal ester (**215**) with acid led to hemiketal (**214**) and α,β-unsaturated ester (**216**), as did acid treatment of ketal (**213**). Consequently, there remains the possibility that all components, other than the peroxy acids and esters, are artefacts.

In 1983, Rinehart described the characterization of peroxy acids from two Caribbean sponges.[56] From an unnamed genus of the family *Plakinidae*, two peroxy acids, with strong antifungal activity were shown to be **218** and **219**, called plakinic acids A and B. The ring stereochemistry for acid B is based on NMR spectral analysis of the methyl ester acquired from ozonolysis-oxidation of the natural acid.

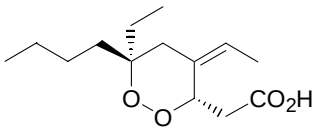



(**218**) plakinic acid A

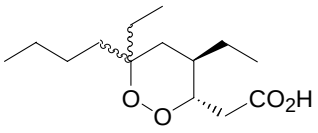



(**219**) plakinic acid B

Cytotoxic peroxy acids have been described from a sponge *Callyspongia* sp. (family *Callyspongidae*) from New Guinea, with the major component being the unsaturated peroxy acid (**220**).[61] Relative stereochemistry followed from orthodox NMR spectroscopic arguments, and both acids inhibited murine leukemia cell growth.



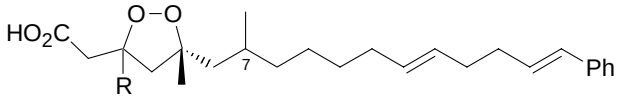

(**220**) major



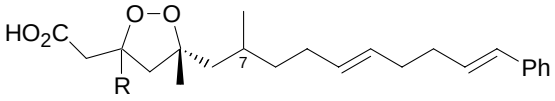

(**221**) minor

A *Plakortis* sp. collected near Fiji, was shown by Davidson to be a source of the less common

1,2-dioxolane type of peroxy acids, with the characterization of plakinic acids C and D, and *epi*-plakininc acids C and D.[17]



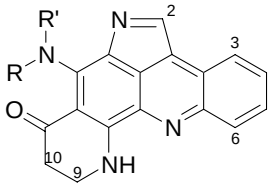
(**222**) plakinic acid C R = β-Me (*cis*)
(**223**) *epi*-plakinic acid C R = α-Me (*trans*)



(**224**) plakinic acid D R = β-Me
(**225**) *epi*-plakinic acid D R = α-Me

Assignment of C-3 as the epimeric centre is arbitrary, and the relative stereochemistry at C-7 is unknown. The new acids and methyl esters exhibited cytotoxicity against human epidermoid carcinoma (KB) cells, human colorectal adenocarcinoma cells and L1210 murine leukemia cells, and full data are available.

A Vanuatan red sponge in the genus *Plakortis* provided two novel pentacyclic aromatic alkaloids – plakinidine A and B – that exhibited *in vitro* activity against the parasite, *Nippostrongylus brasiliensis*.[62]

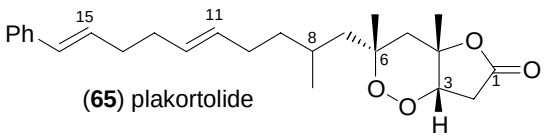


(**226**) plakinidine A R = H, R' = Me
(**227**) plakinidine B R = R' = Me
(**228**) plakinidine C R = H, R' = Me, 9,10-didehydro
(**229**) plakinidine D R = R' = H

Only plakinidine A was active against reverse transcriptase. The purple color of the plakinidines is pH-dependent and is similar to color changes observed for the polycyclic aromatic alkaloids from other marine organisms. The plakinidines were the first structures having the pyrrolo[2,3,4-*kl*]acridine nucleus.

The structural elucidation of the plakinidines A and B was also reported at about the same time by Ireland and Clardy,[63] from a Fijian sponge source. Three colored alkaloids plakinidines A, B, and C were acquired, with the latter being a 9,10-dehydro version of plakinidine A. Subsequently, plakinidine D was isolated from an undescribed *Didemnum* sp. ascidian from Indonesia and Palau.[64]

Another *Plakortis* sp. collected around Fiji yielded a peroxylactone, called plakortolide,[15] which has some structural resemblance (peroxide ring fused to a lactone) to a compound previously isolated by the Faulkner group.[3] Plakortolide displayed moderate cytotoxicity towards human epidermoid carcinoma (KB) cells, colorectal adenocarcinoma and L210 murine leukaemia cells ($ID_{50}$ 0.34 μg/mL).

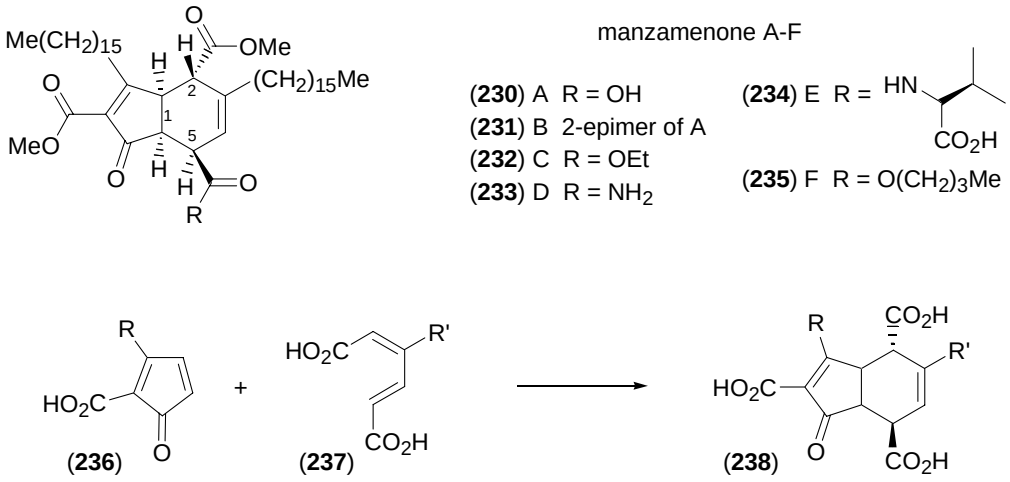


(**65**) plakortolide

Kobayashi has described some very unusual metabolites from an Okinawan *Plakortis* sp.,[65] and related them biosynthetically. The manzamenones A-F (**230**-**235**) are especially interesting, and represented a previously unknown carbon skeleton.

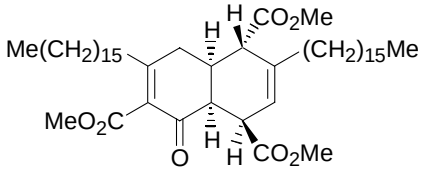
Manzamenone A showed no cytotoxicity against L1210 murine leukemia cells. Biosynthetic speculation was that the manzamenones were derivable from two fatty acid derived systems engaging in an

(enantioselective) *endo* [4+2] cycloaddition as shown below.

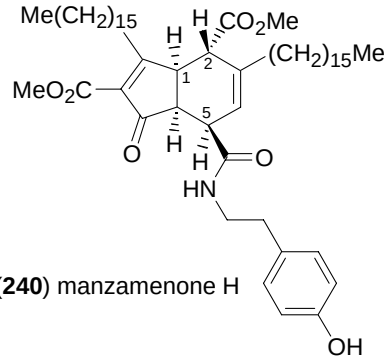



Scheme 23

A subsequent report described the isolation of two additional members – manzamenones G and H – from an Okinawan *Plakortis* sp.[66] The tyramine unit was shown to be attached to the C-5 carboxyl group, by the preparation of manzamenone H from manzamenone A and tyramine hydrochloride. Again, these bicyclo[4.4.0]decane and bicyclo[4.3.0]nonane containing systems may result from intermolecular cycloadditions of fatty acid generated precursors.

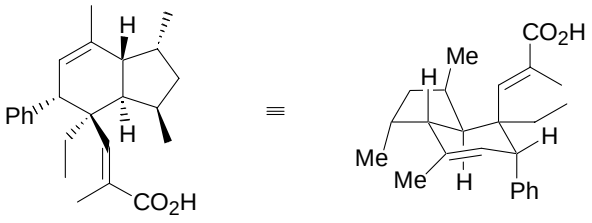


(**239**) manzamenone G



(**240**) manzamenone H

A carboxylic acid with cytotoxic activity was also isolated from an Okinawan sponge, *Plakortis* sp.[67] The structure and relative stereochemistry were based on extensive spectroscopic data and shown below, **141**.

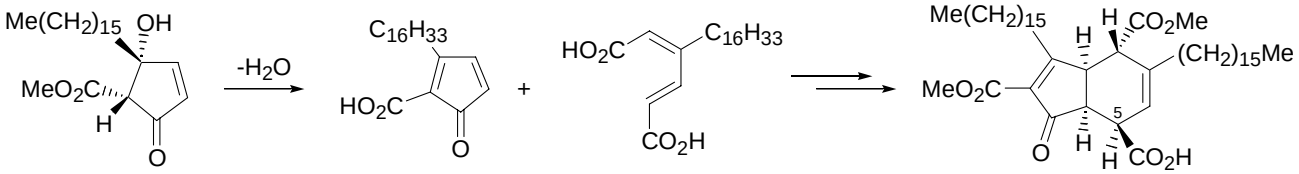


(**141**) plakotenin

Scheme 24

Again, the acid plakotenin is notionally derivable from a linear polypropionate system, clearly allied to the plakinic acids, by intramolecular [4+2] cycloaddition, as proposed for ircinianin, also a sponge derived furanosesterterpene.

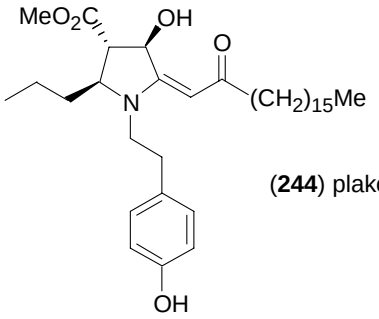
Subsequent support for these biosynthetic postulates emerged with the characterization of a cyclopentenone derivative, untenone (**241**), from the Okinawan *Plakortis* sp.[68] This enone corresponds to the dienophile in the postulated scheme summarized earlier. Untenone A (**241**) and manzamenone A (**230**) have been shown to inhibit mammalian DNA polymerases α and β and also human terminal deoxynucleotidyl transferase (TdT).[69]




(**241**) untenone (**242**) (**243**) (**230**) manzamenone A

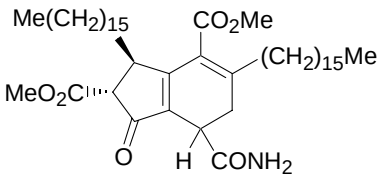
Scheme 25

A pyrrolidine alkaloid, plakoridine A, incorporating some of the sub-structures of the manzamenones, was also isolated from the Okinawan sponge *Plakortis* sp.,[70] with the relative stereochemistry shown below. The hexadecanyl side chain is found in the manzamenones, and untenone, and a tyramine unit was featured in manzamenone H. Plakoridine A was only weakly cytotoxic against murine lymphoma L1210 cells *in vitro* ($IC_{50}$ 10 μg/mL).

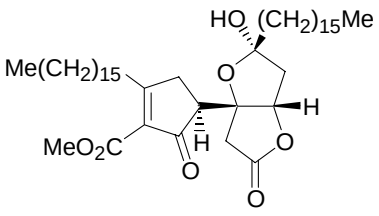



(**244**) plakoridine A

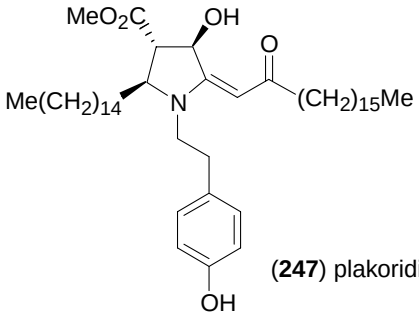
The metabolic richness of Okinawan *Plakortis* sp. was further demonstrated by the characterization of manzamenones J and K and plakoridine B,[71] three new oxylipins with unique skeletons related to 3,6-dioxo-4-docosenoic acid.




(**245**) manzamenone J



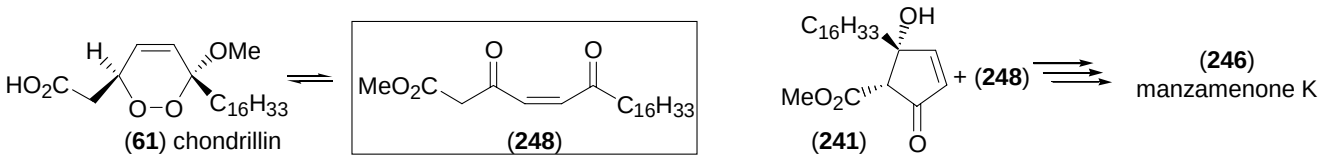

(**246**) manzamenone K




(**247**) plakoridine B

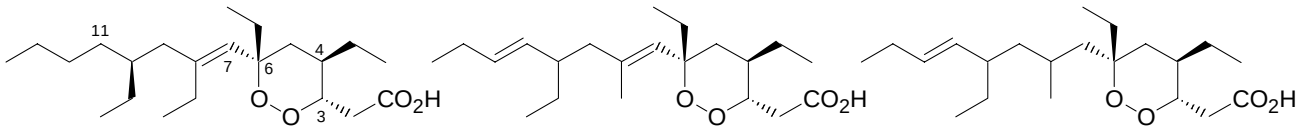
Manzamenone A (**230**), the most abundant constituent incorporating a bicyclo[4.3.0]nonane framework, was deduced to have (5*R*) configuration. Further consideration of the biosynthesis of several *Plakortis* metabolites led to the suggestion that 3,6-dioxo-4-docosenoic acid (ester) (**248**) may be a key

intermediate, as shown below. More details may be found elsewhere.[71]



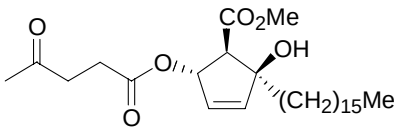

Scheme 26

The Kobayashi group characterized additional structural types from the Okinawan *Plakortis* sp.[72] A series of cyclic peroxides related to known sponge metabolites were described (**249**)-(**251**), with the absolute stereochemistry indicated.



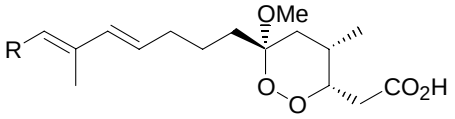

(**249**) (**250**) (**251**)

A second report described a cytotoxic oxylipin, plakevulin A,[73] incorporating a cyclopentene and a levulinyl ester. The absolute stereochemistry was determined by analyses of a reduction product and a modified Mosher's method. Plakevulin A exhibited inhibitory activity against DNA polymerases α and γ, and bears close structural similarity to untenone A (**241**), a possible precursor of the manzamenones.



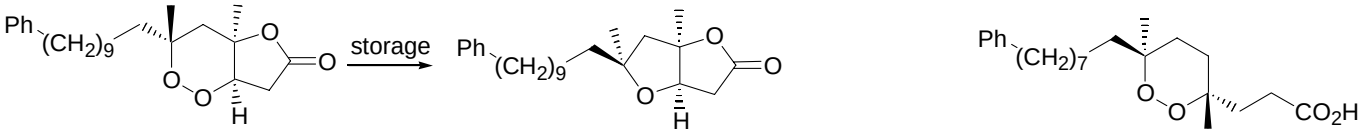

(**252**) plakevulin A

An Indonesian sourced *Plakortis* sp. yielded two cytotoxic 3,6-epidioxy fatty acids which were moderately active against various anti-tumor cell lines.[74] The absolute stereochemistry was based on chemical modification followed by analyses of MTPA esters.




(**253**) R = Me manadic acid A
(**254**) R = Et manadic acid B

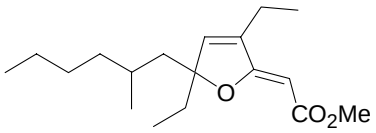
Crews and co-workers described additional peroxy-containing metabolites, plakortolide E and plakoric acid from fractions of a Fijian *Plakortis* sp.,[75] which were active *in vitro* against melanoma cancer cells. Some samples of plakortolide E, on long storage, underwent a ring contraction (with formal oxygen atom loss) to the plakortolide ether. Plakortolide E showed selective potency against the melanoma and breast tumor cell line in the *in vitro* 60-cell line panel of the NCI.



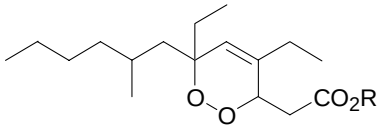

(**255**) plakortolide E (**256**) plakortolide ether (**257**) plakoric acid
(Relative stereochemistry)

Scheme 27

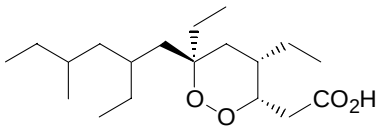
A methanolic extract of a *Plakortis* sp. (*Plakinidae*) collected near the Amirantes Islands was processed to deliver four polyketides whose structures were determined by detailed NMR spectroscopic studies.[76]




(**258**)



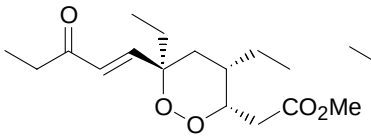

(**259**) R = Me (**260**) R = Et



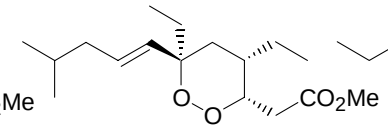

(**261**)

Of the four metabolites, the dihydrofuran and free peroxy acid exhibited toxicity towards *Artemia* larvae. The reasonable view was that the variety of oxygenated polyketides from the genus *Plakortis* may arise by oxidation at the carboxyl end of long-chain branched or unbranched fatty acids, with γ-lactones, ethers and peroxides often representing the oxidation pattern. This may imply a remarkable ability of sponges to utilize a general pathway to generate a wide variety of secondary metabolites, as a function of their needs in differing environments.

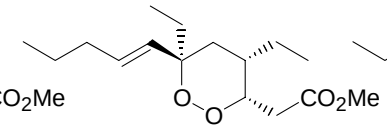
A Jamaican *Plakortis* sponge was examined by Hamann and co-workers,[77] and cyclic peroxides – the plakortides M, N, K, and L – together with plakortone G and the known filiformin, and plakortide F were characterized.



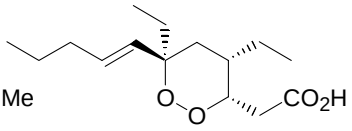

(**99**) plakortide M



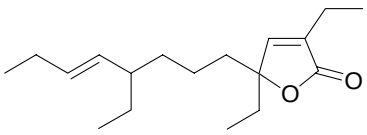

(**262**) plakortide N



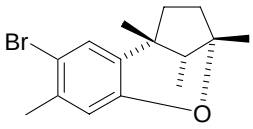

(**100**) plakortide K



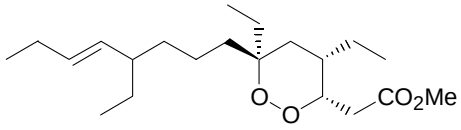

(**263**) plakortide L




(**264**) plakortone G



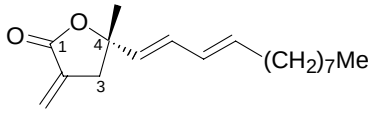

(**265**) filiformin




(**34**) plakortide F

Plakortide M exhibited significant antimalarial activity. The actual origin of filiformin was unclear. The new plakortone G varies from other lactones from *Plakortis* sp. by lacking a tetrahydrofuran moiety. It was highly cytotoxic but exhibited little selectivity towards a particular tumor cell type. The general activity of the peroxy compounds from the genus *Plakortis* makes this class of sponges worthy of close evaluation, with careful storage and extraction procedures, in the quest for effective anti-malarial agents.

A new γ-lactone, plakolide A, from *Plakortis* sp., was recently reported,[78] and found to inhibit inducible nitric oxide synthase (iNOS) activity. New structural features were α-exomethylene and γ-disubstituted patterns in the γ-lactone. The (4*S*) configuration was based on CD data.

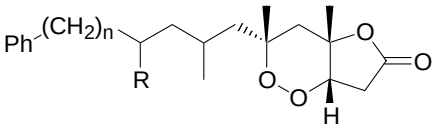



(**266**) plakolide A

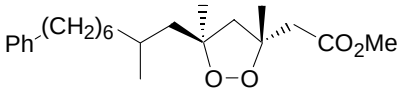
## METABOLITES FROM *PLAKINASTRELLA* SPECIES

*Plakinastrella onkodes*

The first examination of *Plakinastrella onkodes* (from the Gulf of Mexico) was reported in 1994,[79] after it was noted that an extract exhibited cytotoxicity in whole-cell essays against certain lung carcinoma and leukemia cell lines. Three new peroxylactones, the plakortolides B, C, and D, the methyl ester of *epi*-plakinic acid E, and a mixture of steroidal peroxides were isolated. The relative stereochemistry is the same as that deduced for plakortolide itself (**65**).[15]



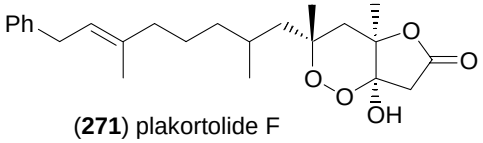
(**267**) plakortolide B R = H, n = 6
(**268**) plakortolide D R = Me, n = 6
(**269**) plakortolide C R = H, n = 4
(**65**) plakortolide R = H, $\Delta^{11,15}$, n = 6



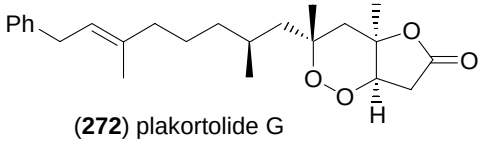
(**270**) *epi*-plakinic acid E methyl ester

Isolated plakortolides B and D and *epi*-plakinic acid (ester) were found to show cytotoxicity towards the A549 human lung carcinoma and P388 murine leukemia cell lines.

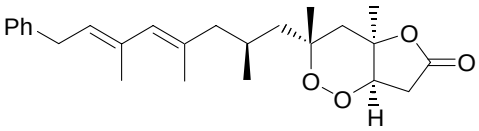
Further plakortolides F and G (along with the parent plakortolide (**273**))[80] were characterized from a Jamaican sample of *Plakinastrella onkodes*.[81] The displayed absolute stereochemistry for plakortolide G was based on *ab initio* computations of optical rotations.



(**271**) plakortolide F



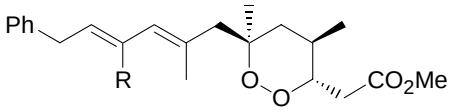
(**272**) plakortolide G



(**273**) plakortolide

The parent plakortolide (**273**) and plakortolide G (**272**) exhibited potent inhibitory activity against the protozoan *Toxoplasma gondii*, which belongs to a group which causes morbidity and mortality in immunocompromised patients.

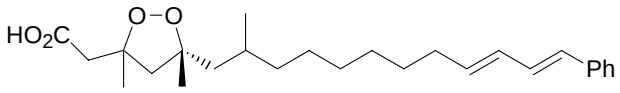
*Plakinastrella onkodes* provided a new cytotoxic peroxide, methyl capucinoate (**274**), and a known, but incompletely defined peroxide (**275**).[9] These cyclic peroxides showed *in vitro* cytotoxicity, but no *in vivo* activity against murine leukemia P388.




(**274**) methyl capucinoate, R = H
(**275**) known peroxide, R = Me

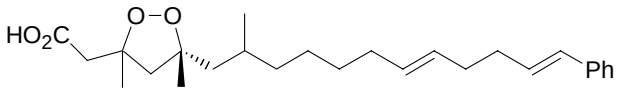
(The methyl esters may be artifacts from methanol extraction)

The sponge *Plakinastrella onkodes*, from Sanibel Island, Florida, led to the new aromatic acids (**276**)-(**277**).[10]




(**276**) R = α-Me (**277**) R = β-Me

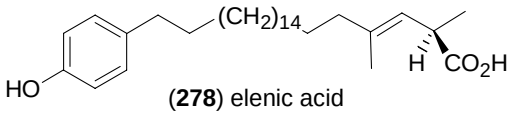
Compare with:




(**222**) plakinic acid C (β-Me) and (**223**) *epi*-plakinic acid C (α-Me) (see ref. 17)

*Plakinastrella* sp.

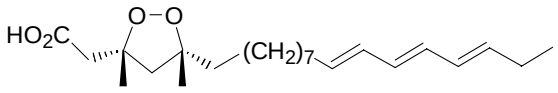
In 1995, Scheuer reported the isolation of elenic acid [(*R*)-2,4-dimethyl-22-(*p*-hydroxyphenyl)-docos-3(*E*)-enoic acid] from an Indonesian *Plakinastrella* sp.[82]



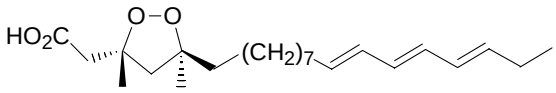

(**278**) elenic acid

The (2*R*)-configuration was determined by utilizing amide formation with (*R*)- and (*S*)-phenylglycinyl methyl esters. This acid was cytotoxic with $IC_{50}$ of 5 μg/mL in P-388, A-549 and MEL-28 bioassays, and inhibited topoisomerase II (indicator enzyme in the treatment of lung cancer) at 0.1 μg/mL, comparable with actual clinical agents, of more complex structure. Elenic acid has also been synthesized by Mori (see below).[106] Further detailed studies of the biological activity of elenic acid have confirmed[83] it to be a DNA topoisomerase II inhibitor, of calf DNA polymerase α ($IC_{50}$ 7.7 μM) and rat DNA polymerase β ($IC_{50}$ 12.9 μM).

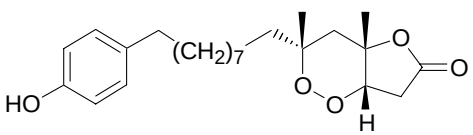
A *Plakinastrella* sp. collected off the Seychelles provided an ethanol extract that was found to inhibit the growth of the fungal pathogens *Candida albicans* and *Aspergillus fumigatus*.[84] Bioassay-guided purification resulted in two new 1,2-dioxolane acids, incorporating a conjugated triene in a side-chain, along with a peroxide-lactone, plakortolide F (**281**)[85] incorporating a *p*-phenol linked by a saturated chain.




(**279**) plakinic acid F

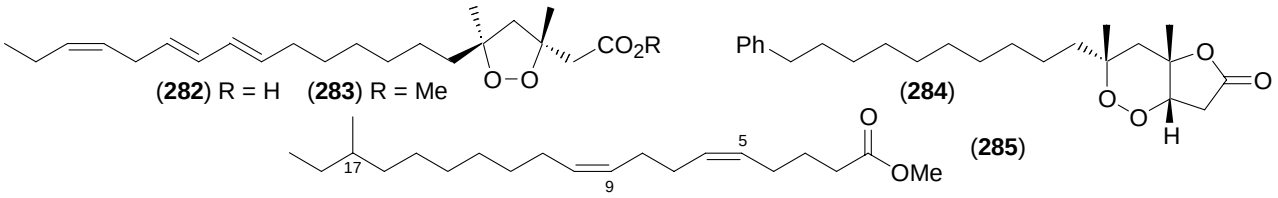



(**280**) *epi*-plakinic acid F




(**281**) plakortolide F

A *Plakinastrella* sp. from the Philippines has been examined by the Faulkner group, and the isolation of three new cyclic peroxides and a carboxylic acid was described.[16a] (This species is taxonomically close to other *Plakinidae* that provide peroxide-containing metabolites.)



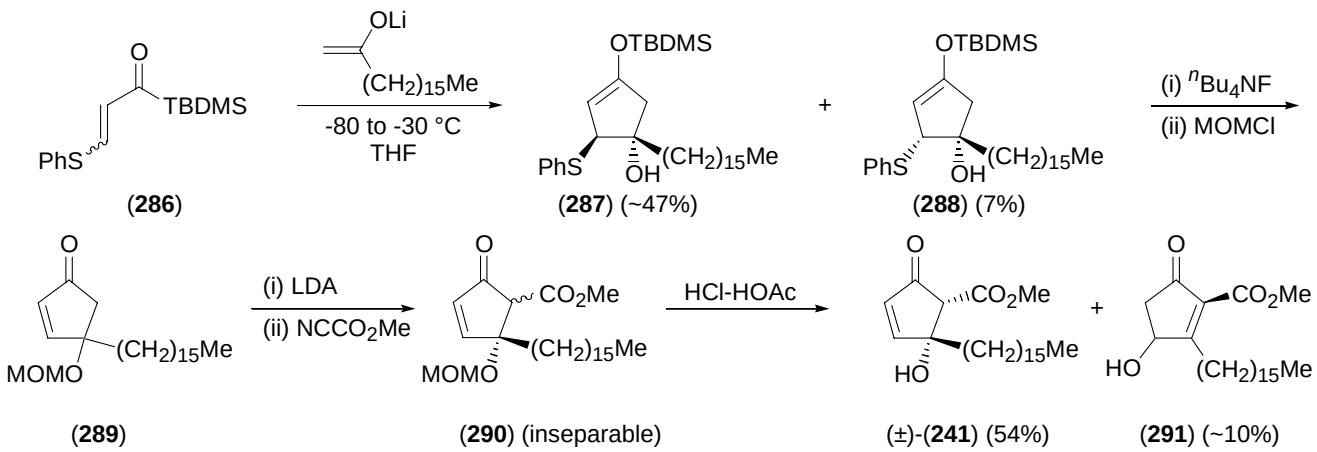

The structure assigned to the dienoate (5*Z*,9*Z*)-17-methylnonodeca-5,9-dienoate (**285**), was shown by synthesis by Mori, to be misassigned, with the *Plakinastrella* metabolite being a mixture of two esters, neither of which was the originally deduced structure. Subsequently, Mori reported the synthesis of the (*R*)-enantiomer of methyl (5*Z*,9*Z*)-17-methylnonodeca-5,9-dienoate,[86] the structure initially proposed by Faulkner, and its NMR parameters differed from those of the *Plakinastrella* sp. metabolite.

## SYNTHESES OF *PLAKORTIS* METABOLITES

In the foregoing discussions of the metabolites from *Plakortis* sp., some allusions to synthesis were made, but it was considered desirable to present this aspect in a separate section. Synthetic efforts towards these novel structural types have been increasing and are of considerable interest.

*Syntheses of Untenone A and Manzamenones*

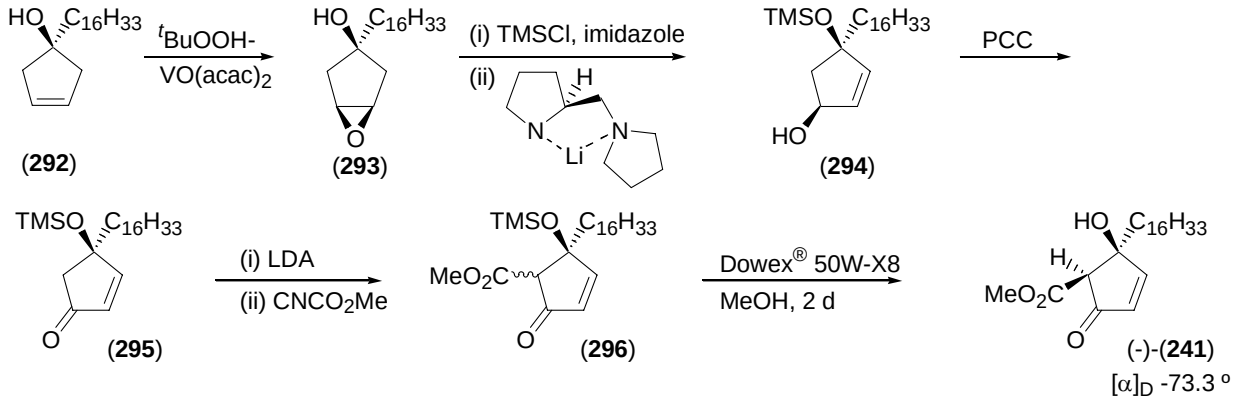
It is convenient to discuss these metabolites jointly as the cyclopentenone untenone is considered to be a biosynthetic precursor of the manzamenone system. In 1994, Yoshii described an approach to racemic untenone A,[87] the key step of which was a [3+2] annulation of β-(phenylthio)acroylsilane with an enolate of 2-octadecanone. This was followed by α-methoxycarbonylation to afford mainly (±)-untenone A (**241**).




Scheme 28

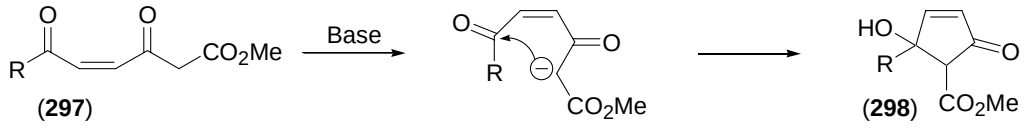
This synthesis, although in the racemic series, and affording isomers, confirmed the assigned structure (by Kobayashi)[68] of untenone A (**241**) and relative stereochemistry.

Subsequently, Asami synthesized (–)-untenone A with the key step being enantioselective deprotonation of a *meso*-epoxide.[88] The optical rotation of natural untenone A was reported as $[\alpha]_D$ +0.2 º (c, 2.1, $CHCl_3$),[68] but the optical purity and absolute configuration were unknown. Hydroxy-directed epoxidation of a cyclopentenol afforded an epoxide which, after protection experienced enantioselective deprotonation, with lithium (*S*)-2-(pyrrolidin-1-ylmethyl)pyrrolidide, and allylic alcohol formation. Standard manipulation led to (–)-untenone, $[\alpha]_D$ –73.3 º ($CHCl_3$), mp 62-64 ºC.



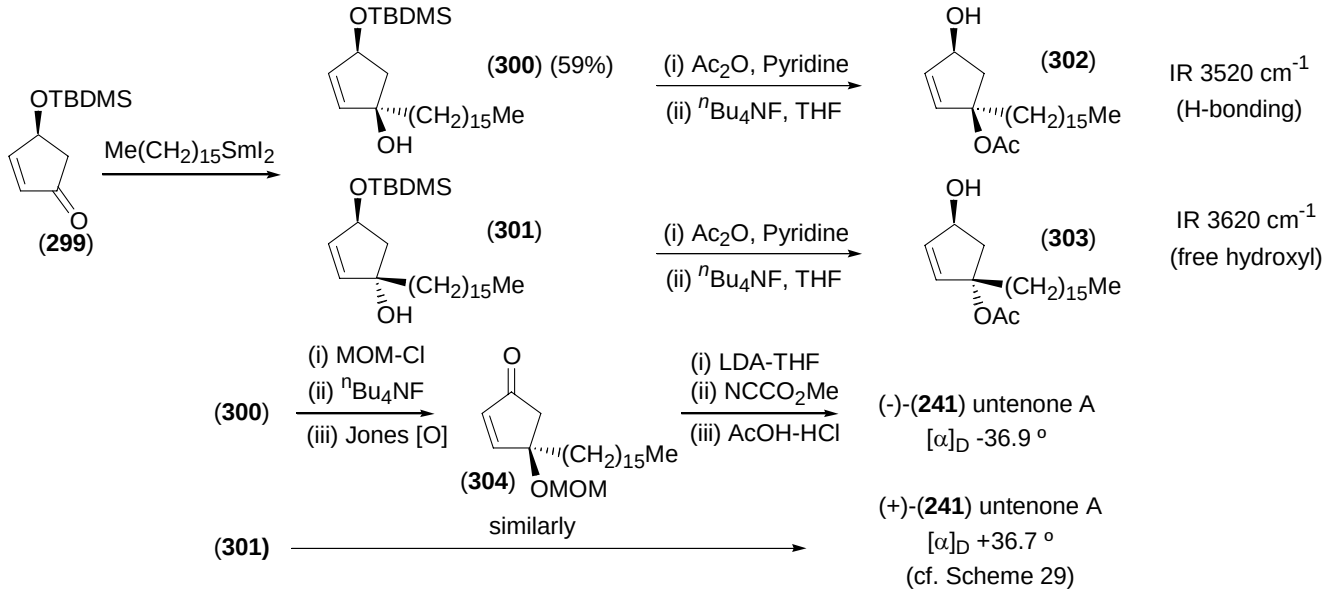

Scheme 29

The very low rotation reported for the natural product, and the substantial value for (–)-untenone, suggest the natural compound is racemic and probably arises from base-induced cyclization of a 1,4-diketo ester.



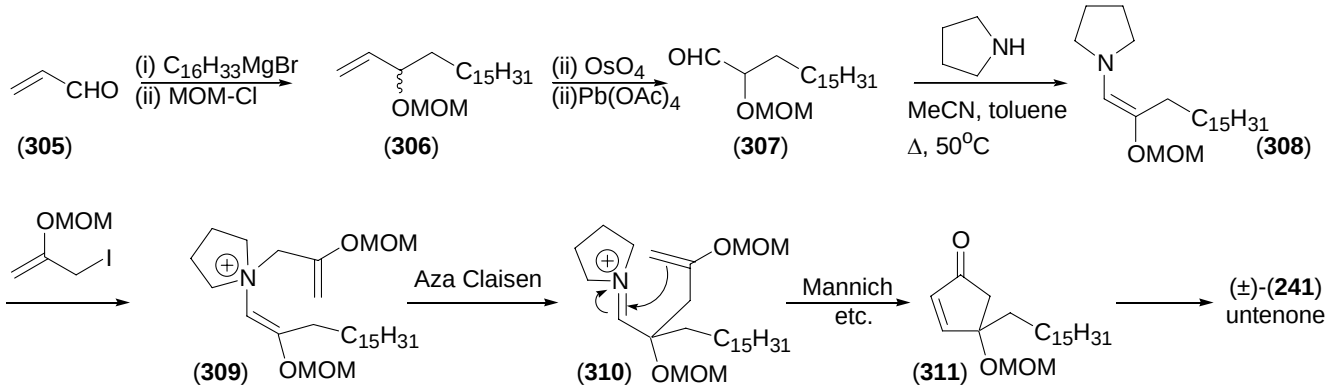

Scheme 30

That natural untenone was in fact racemic, was shown by Yamada,[89] who synthesized both enantiomers and then compared their CD spectra with that of the natural compound, which lacked the Cotton effect. (*S*)-4-[(*tert*-Butyldimethylsilyl)oxy]-2-cyclopentanone acquired from L-(+)-diethyl tartrate, was the starting material, and its conversion to (+)- and (–)-untenone is shown below.



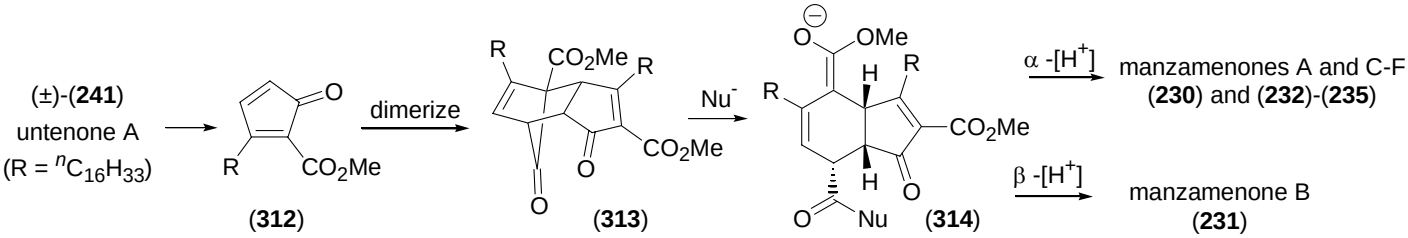

Scheme 31

Routes to 4-alkyl-4-hydroxy-2-cyclopentenone systems by [3+2] annulation, were explored by Florent and co-workers,[90] who executed a formal total synthesis of (±)-untenone.



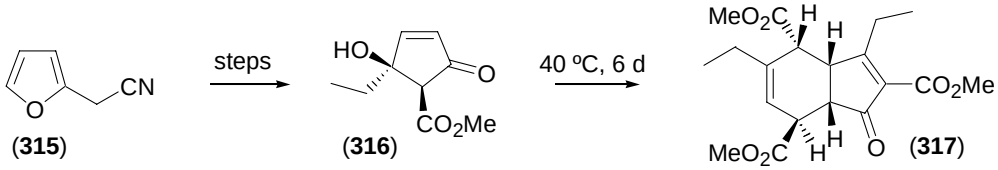

Scheme 32

Whitehead and co-workers developed an approach to the manzamenone system on the basis that a reactive cyclopentadienone may initially dimerize to yield a bridged tricycle which may transform to a functionalized bicyclo[4.3.0]nonane, the characteristic feature of the manzamenones.[91] This hypothesis is summarized below, with the natural occurring (±)-untenone A (**241**) representing a precursor of the cyclopentadienone.




Scheme 33

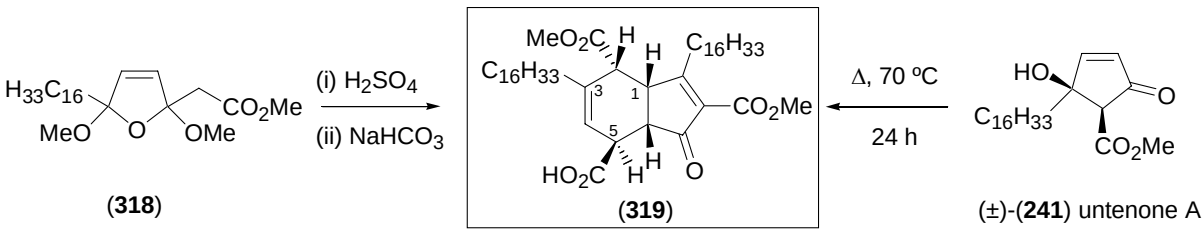
This hypothesis formed the basis of a synthetic approach, which began with the synthesis of the ethyl analogue of (±)-untenone A from 2-furanoacetonitrile, by substitution and oxidative rearrangement. Heating a neat liquid sample of the cyclopentenone formed a single product, possessing the desired [4.3.0]-nonane skeleton, and bearing two esters and one carbonyl group. The relative stereochemistry was confirmed by an X-Ray crystallographic analysis. NMR spectral comparisons strongly indicated stereochemical correlation between the synthesized ethyl-analogue and manzamenone A, but different from manzamenone B.




(**315**) (**316**) (**317**)

Scheme 34

This approach was subsequently applied to the natural system of (±)-untenone,[92] with *n*-$C_{16}H_{33}$ instead of the ethyl analogue. Heating (±)-untenone at ≈ 70 ºC furnished a compound whose NMR data were nearly identical with those for the ethyl system, strongly implying the relative stereochemistry below.



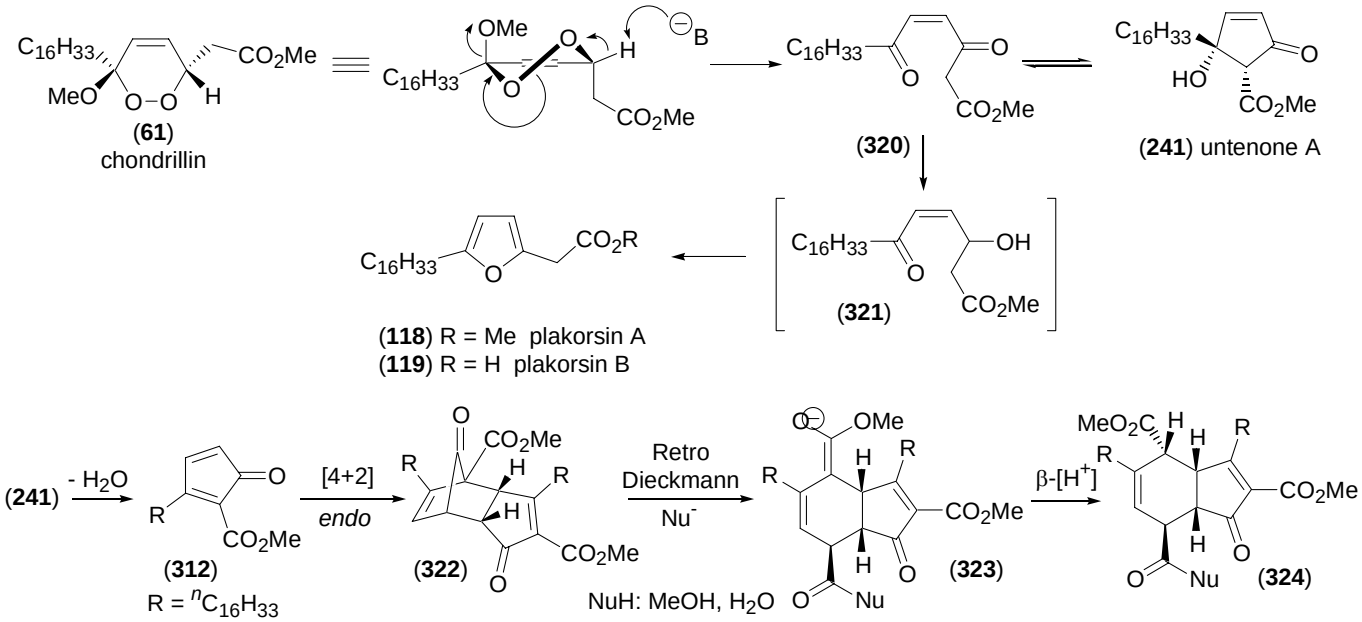

(**318**) (**319**) (±)-(**241**) untenone A

Scheme 35

In fact, this synthesized material (**319**), the ethyl and *n*-hexyl analogue and manzamenone A have the same relative stereochemistry. That is, **319** is manzamenone A. Consequently, the stereo-structure previously proposed for manzamenone A (**230**) should be revised to that of **319** above. Synthetic **319** was esterified to the butyl and ethyl esters (i.e. at the 5-carboxyl group), and these were identical with the natural manzamenones F and C, respectively. Consequently a re-assignment of relative stereochemistry of these manzamenones is also necessary.

It is worth emphasizing that conversion of the bis-ketal above to manzamenone A (**319**), by a two-step sequence (acid and then base) induces sequentially acetal hydrolysis, aldol cyclization, dehydration, dimerization and retro-Dieckmann reaction.

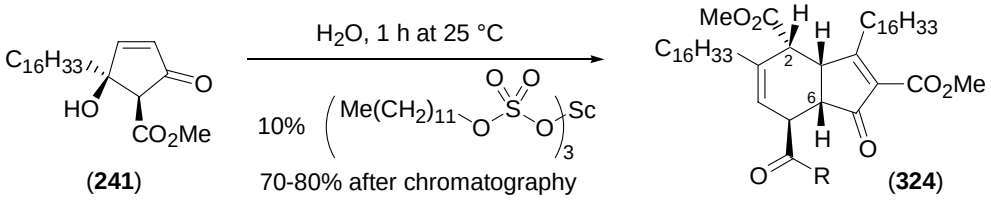
The details of the conversion of untenone A to manzamenone A have also been described,[93] and applied to a number of shorter chain analogues of the natural compouds. The general propositions outlined in the brief reports were supported by further studies, and modification of the original biosynthetic proposal has untenone A arising from chondrillin, which also could provide plakorsin A and B.




Scheme 36

Quenching experiments with methanol and methanol-$d_4$ supported the above proposal as did modeling studies which favored the system that resulted from the synthetic approach. Thus the favored stereostructures for the majority of the manzamenones are in accord with both kinetic and thermodynamic predictions. Overall, Whitehead and co-workers described syntheses of untenone A, manzamenones A, C and F and plakorsins A, B, based on a plausible biosynthetic hypothesis. The relative stereostructures of the majority of the manzamenones need revisions so that the C-2 acyl group is α and that at C-5 is β.

On the basis that the natural conversion of untenone A to manzamenone A illustrated the concept of predisposition in chemical reaction, the cycloaddition should proceed in an aqueous system, particularly in micellar media. Untenone, in water, in the presence of sodium dodecyl sulfate led clearly to manzamenone A, in good yield, but quite high levels of surfactants were needed.[94] Optimization led to the following conditions for the conversion.



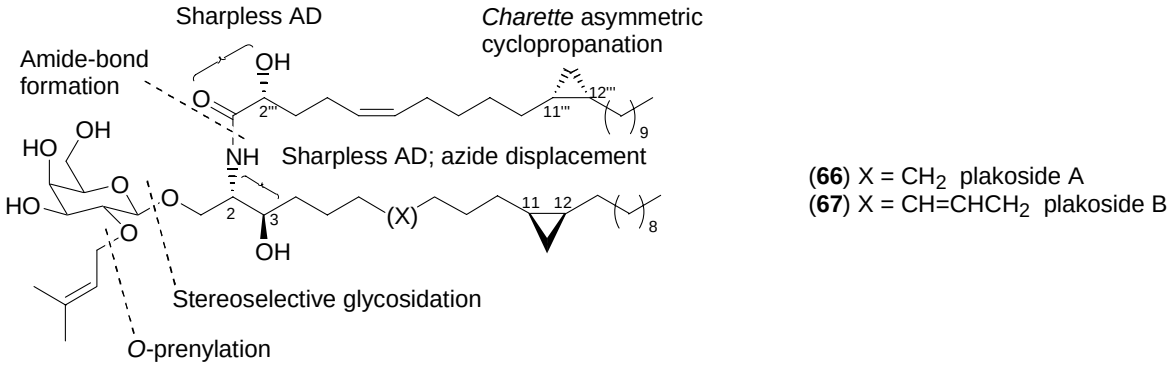

Scheme 37

Use of $D_2O$ led to substantial incorporation at C-2 (and some at C-6), in accord with the mechanism detailed above.

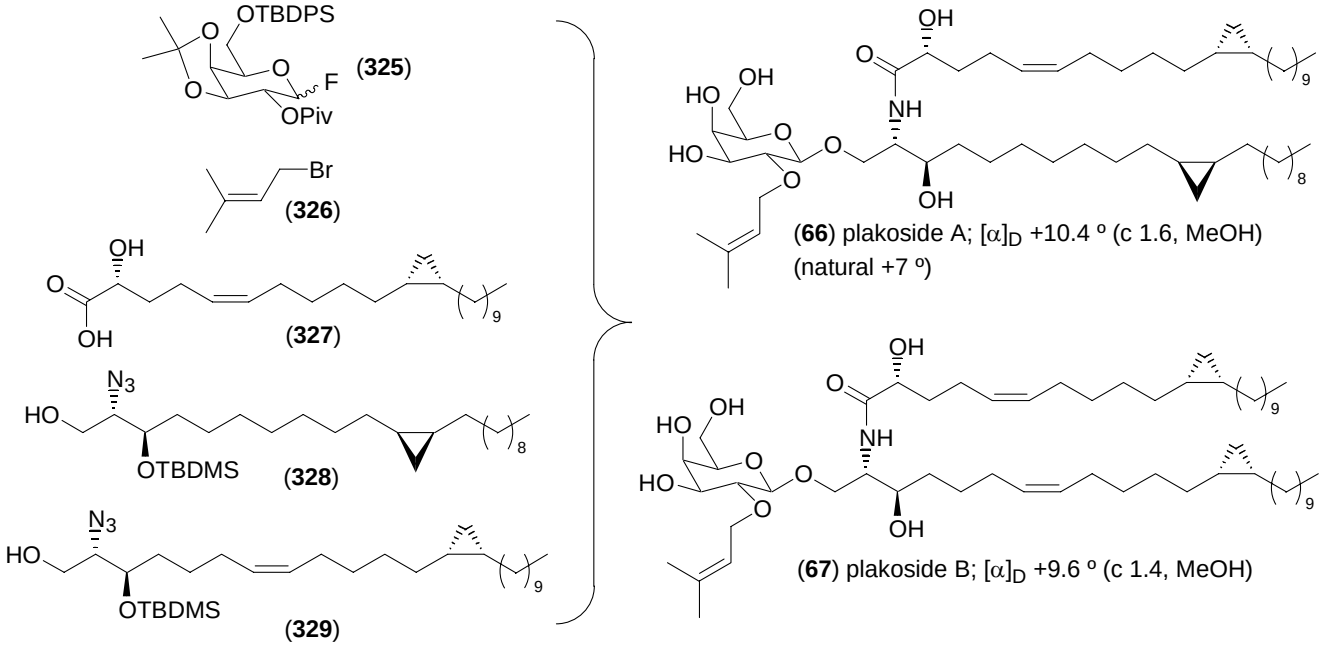
*Syntheses of Glycosphingolipids*

The glycosphingolipids plakosides A and B, isolated from the sponge *P. simplex* possess very unusual structures and present substantial synthetic challenges, particularly with respect to the stereochemistry of the cyclopropane-bearing alkyl side chains.

Nicolaou first reported the synthesis of the plakoside system, and some selected analogues, and the elements of their retrosynthesis are shown below.[95]




The convergent approach required construction of the retrosynthetically indicated buildings blocks, with Sharpless asymmetric dihydroxylation and Charette asymmetric cyclopropanation introducing the desired configurations. In this way, the following building blocks were acquired and then linked by glycosidation and amide bond creation after manipulation and deprotection. In this way, the stereoisomers of plakoside A and plakoside B shown below were obtained.

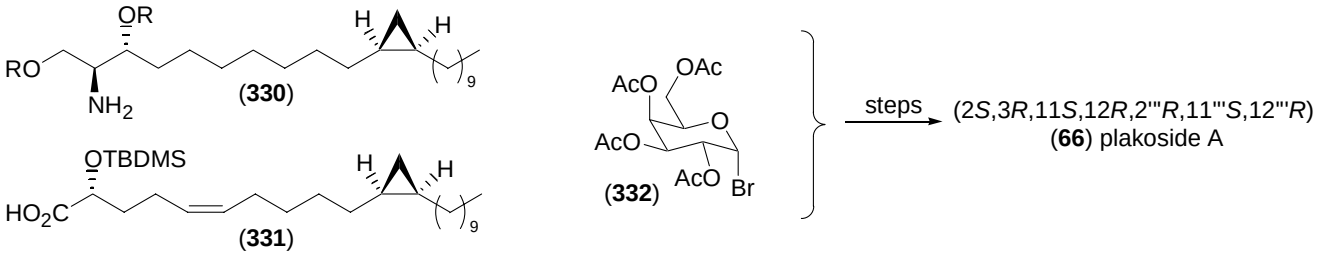



Scheme 38

Both the synthesized compounds exhibited spectroscopic data identical with those reported for the natural compounds, apparently confirming the above absolute stereochemistry for the natural products. The

synthesized compounds and some analogues were found to exhibit only modest immunosuppressive properties.

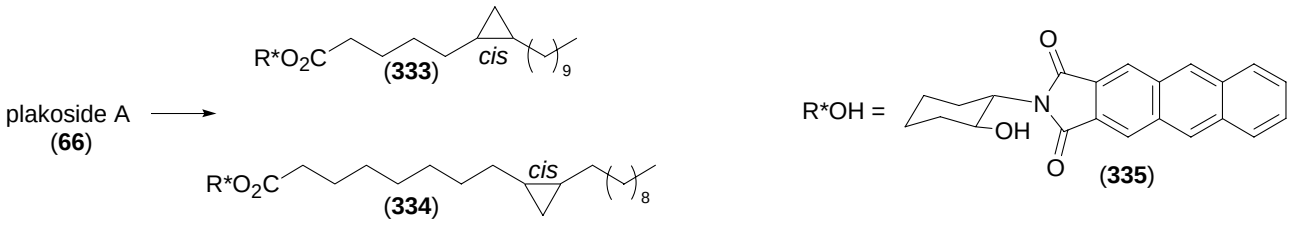
Very shortly after, Mori reported the synthesis of (2*S*,3*R*,11*S*,12*R*,2‴*R*,11‴*S*,12‴*R*)-plakoside A and the (2*S*,3*R*,11*R*,12*S*,2‴*R*,5‴*Z*,11‴*R*,12‴*S*) isomer,[96] with the expectation that one would be the natural product, and that the cyclopropane bearing centers would have the same absolute configuration resulting from the same biocyclopropanation process. The synthetic approach is illustrated by the construction of the sphingosine moiety, the α-hydroxy acid and prenylated sugar to form the (2*S*,3*R*,11*S*,12*R*) isomer mentioned above.




Scheme 39

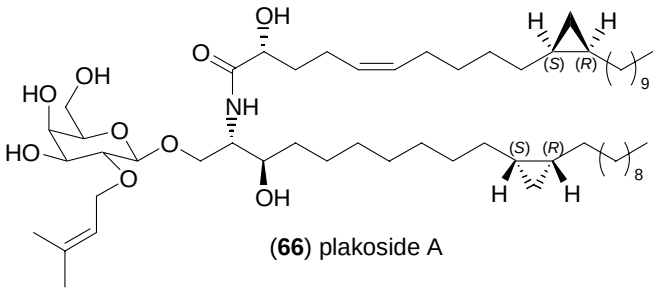
The acquisition of these two diastereomers of plakoside A which were indistinguishable spectroscopically and had similar chiroptical properties, meant that the absolute configuration of the (*cis*)-cyclopropane moiety was not determined. This situation is not too surprising given the relative remoteness of the cyclopropyl sub-unit from others regions of chirality in these molecules.

This outstanding stereochemical question was settled following the degradation of plakoside A to two different cyclopropane containing fatty acids which were derivatized with a chiral agent. HPLC examinations of these and synthetic derivatives of known absolute stereochemistry permitted determination of the absolute stereochemistry as shown below.[97]




Scheme 40

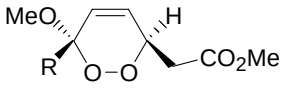
Consequently the complete stereostructure deduced by Mori and Fattarusso is (2*S*,3*R*,11*S*,12*R*,2‴*R*,5‴*Z*,11‴*S*,12‴*R*) and so the isomer synthesized by Nicolaou is a diastereomer of the natural product.



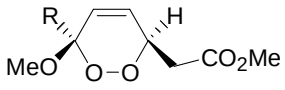
(**66**) plakoside A

*Syntheses of Chondrillin, Plakorin and Xestins*

*Plakortis* sp. metabolites incorporating the structurally novel peroxy moiety were shown to be an important general class, and synthetic efforts towards such compounds have been described. Snider and Shi reported the synthesis of (±)-chondrillin, (±)-plakorin and related peroxyketals.[98] Representative structures are shown below.

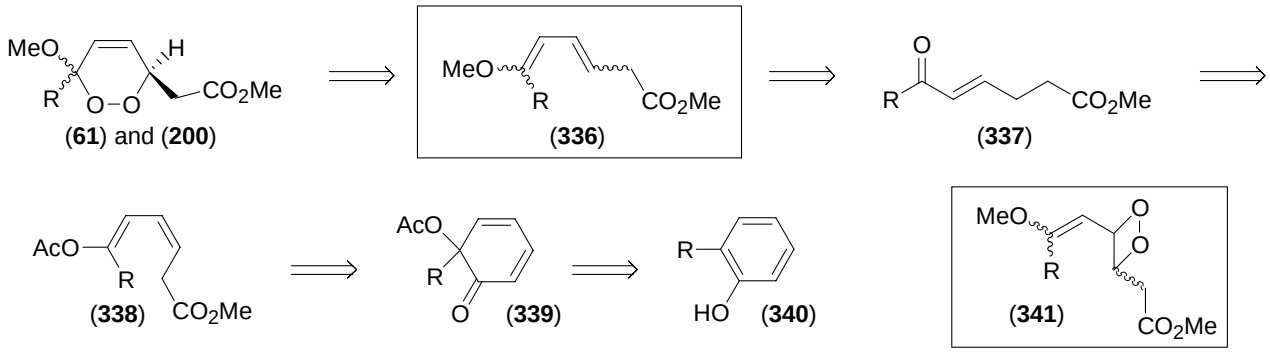


(**61**) R = $^{n}C_{16}H_{33}$ chondrillin
(**60**) R = $^{n}C_{13}H_{26}$-CH=CH-CH=CH-Me xestin B



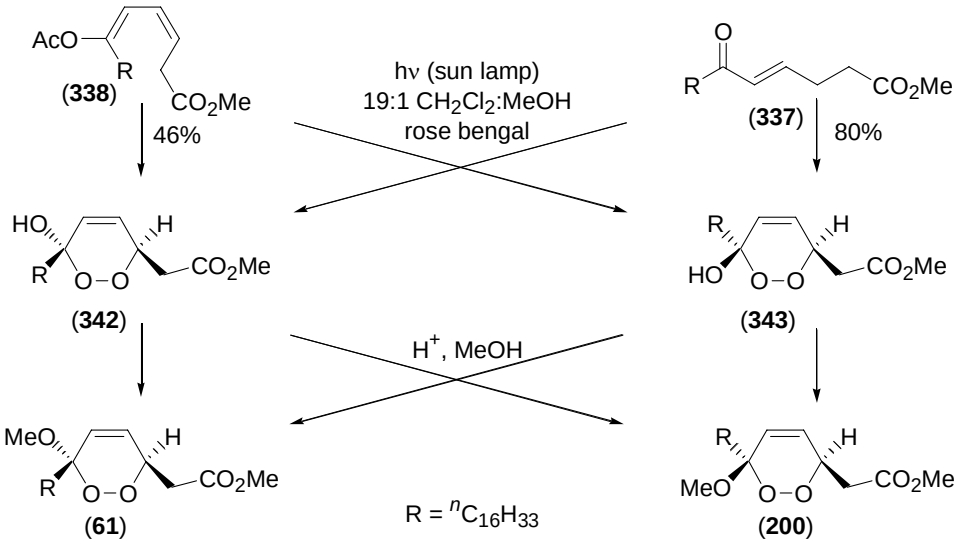
(**200**) R = $^{n}C_{16}H_{33}$ *epi*-chondrillin (plakorin)
(**59**) R = $^{n}C_{13}H_{26}$-CH=CH-CH=CH-Me xestin A

The obvious approach to these compounds was the singlet oxygen Diels-Alder reaction, although existing reports indicated that endocyclic dienes were the most responsive substrates for this reaction. Snider's retrosynthesis is shown below for the general class of peroxy ketals.



Scheme 41

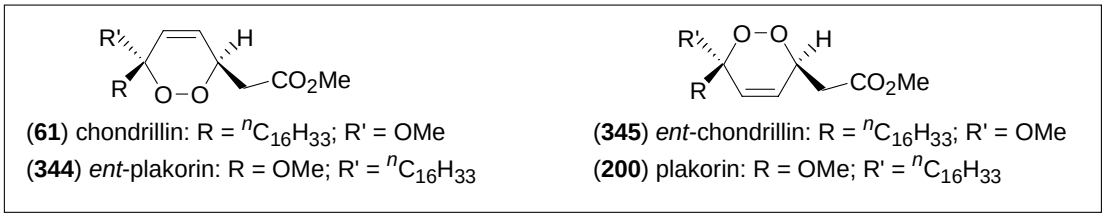
Unfortunately, reaction of the methoxydiene (with singlet oxygen under various conditions) yielded none of the cyclic peroxy ketals, with most products probably originating from the dioxetane (**341**). However, the acetoxydiene reacted with singlet oxygen to provide the corresponding hemiketals as shown below, and these could be separated and are configurationally stable.

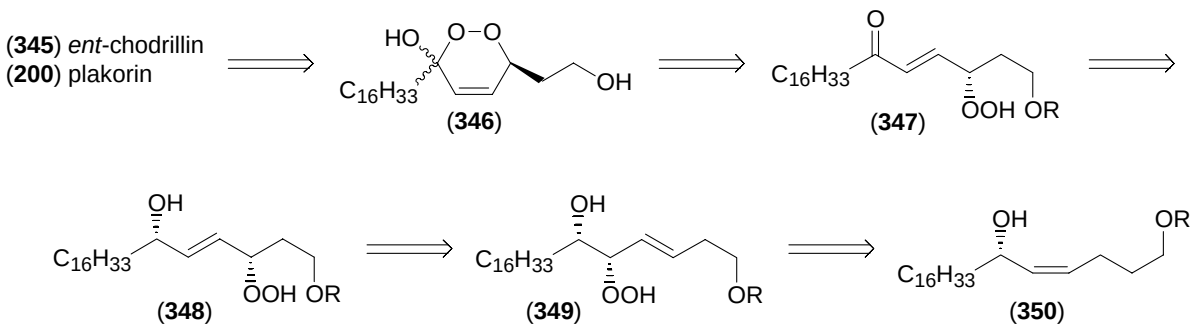



Scheme 42

It appears that the first step in this sequence is photohydrolysis to the enone ester, whose oxygenation is then much more efficient. Overall the conversion from benzene derivative (**340**) to the peroxy ketals proceeds in 28% yields in seven steps. Alkylation, to initially introduce the $C_{16}H_{33}$ and then the above sequence, led to an easily separable mixture of chondrillin (**61**) and plakorin (**200**). Details of the mechanism of peroxy hemiketal formation were investigated, and overall this procedure permits the acquisition of a wide variety of peroxy hemiketals.

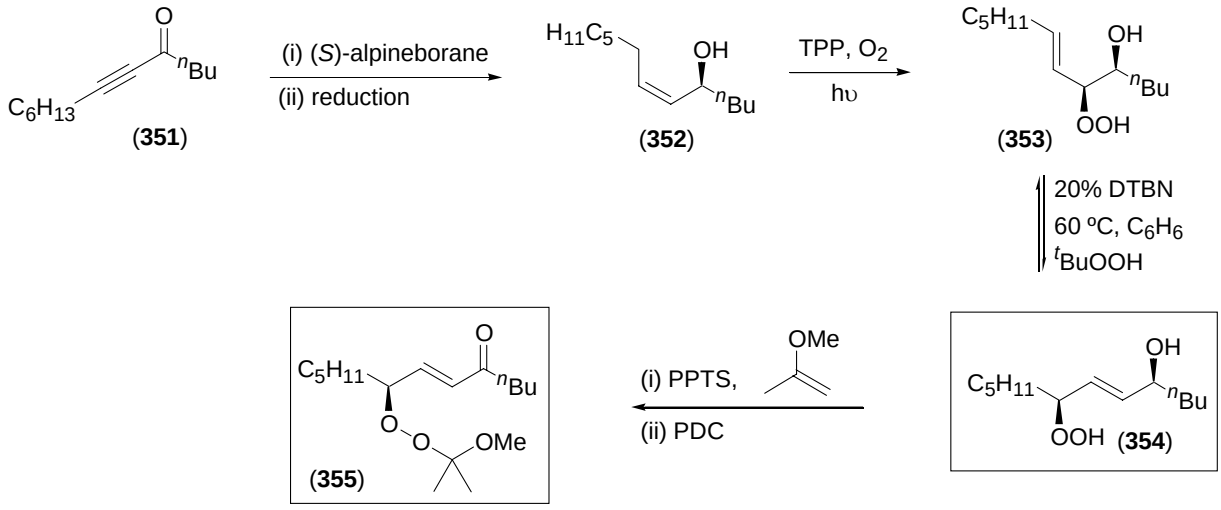
Dussault has also contributed to our understanding of singlet oxygenation as an approach to 1,4-dioxygenated peroxides, and also an asymmetric route to plakorin and *ent*-chondrillin.[99] The initial retro synthesis was based on the reported epimeric relationship between chondrillin and plakorin, and simultaneous formation of the two alkoxydioxines by etherification of epimeric $C_6$-dioxinols.



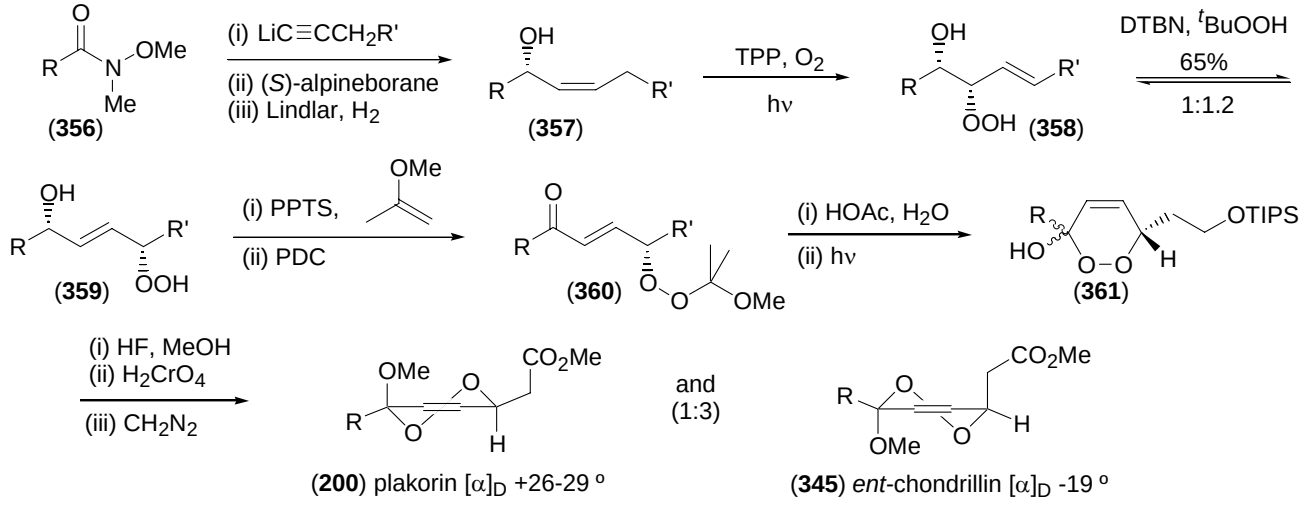

Scheme 43

Model studies led to an approach, summarized below, that seemed applicable to the system that could deliver chondrillin and plakorin. The separable allylic hydroperoxides (recovered in ca 80%) could be protected and oxidized to the peroxyenone, with 87% ee, consistent with stereospecific radical rearrangement.



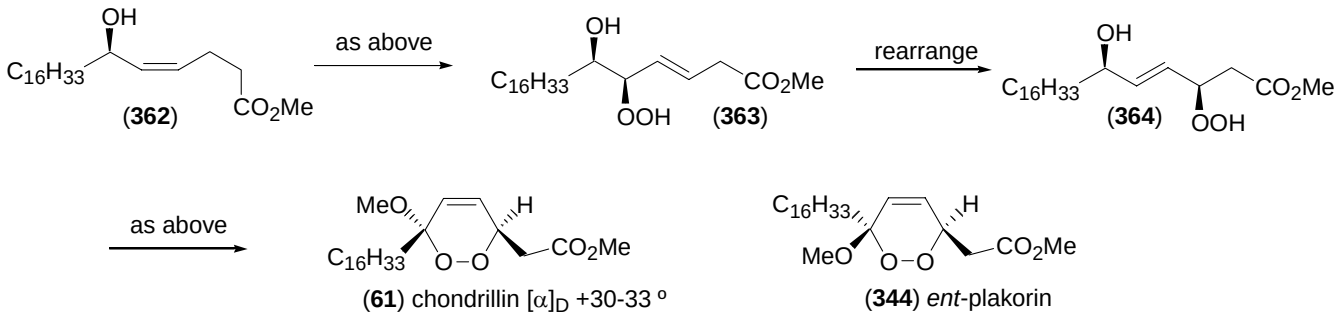

Scheme 44

With respect to the total synthesis of the natural peroxy compounds, the scheme is summarized below.




Scheme 45

Comparisons of these rotations suggested that synthetic plakorin was 85-95% ee, but that the isomer with $[\alpha]_D$ –19 º was opposite in sign to values for natural chondrillin, and therefore was *ent*-chondrillin, and therefore chondrillin is the (3*R*,6*S*) stereoisomer. These conclusions led to development of a more efficient route to chondrillin and *ent*-plakorin, with an important feature being the installation of a (H-bonding) group to effect stabilization of the rearranged 4-hydroperoxyalkenol.



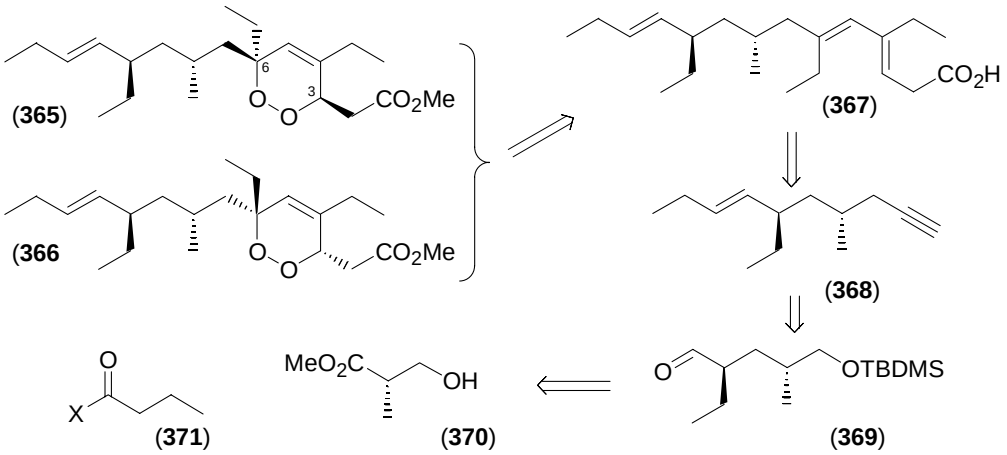

Scheme 46

A full discussion of the likely absolute stereochemistry of chondrillin, plakorin and the xestrins is presented,[99] and in summary the following conclusions are arrived at: chondrillin (3*R*,6*S*) $[\alpha]_D$ +31.5 º; *ent*-chondrillin (3*S*,6*R*) $[\alpha]_D$ –19 º; plakorin (3*S*,6*S*) $[\alpha]_D$ +27.5 º; *ent*-plakorin (3*R*,6*R*) $[\alpha]_D$ –29.9 º.

The results of Dussault[99] are relevant to the mechanism of dioxygenation during biosynthesis. Because the natural compounds are highly enantiomerically enriched, the peroxy moiety appears to be installed by an enzymatic route. Furthermore, because plakorin and chondrillin both have (6*S*) stereochemistry, a feasible route could involve conjugate addition of a hydroperoxy ketal system onto an enoate.

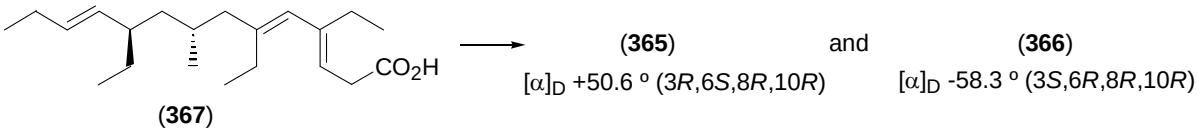
*Syntheses of Miscellaneous Metabolites*

Photooxygenation of a suitable acyclic trienecarboxylic acid has provided access to the plakortic acid family of compounds, particularly the compounds below from *Plakortis angulospiculatus*. Two stereoisomers of these bioactive peroxy acids were acquired utilizing the approach shown below.[100]




Scheme 47

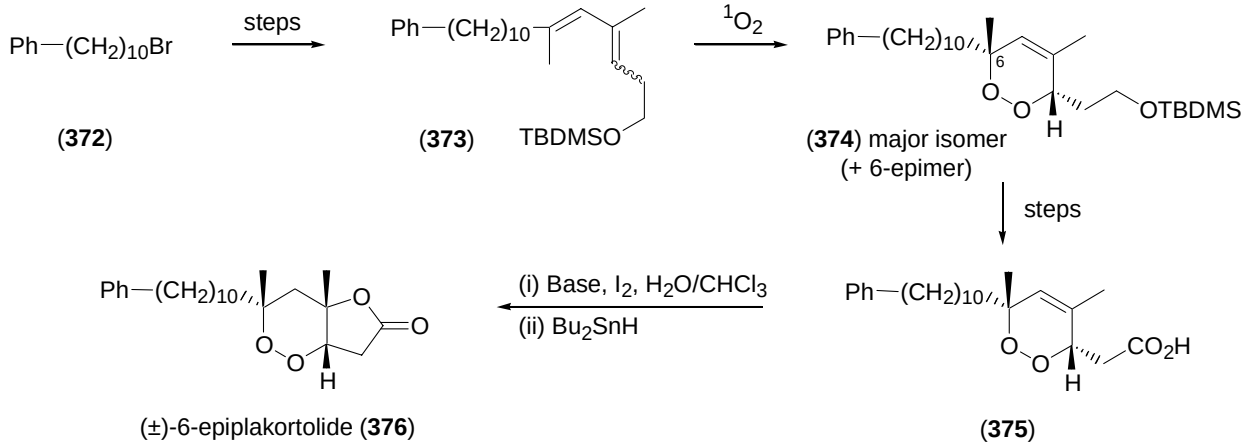
The trienoic acid on reaction with singlet oxygen afforded a diastereomeric mixture of the peroxy carboxylic acids which were converted to the separable methyl esters.




Scheme 48

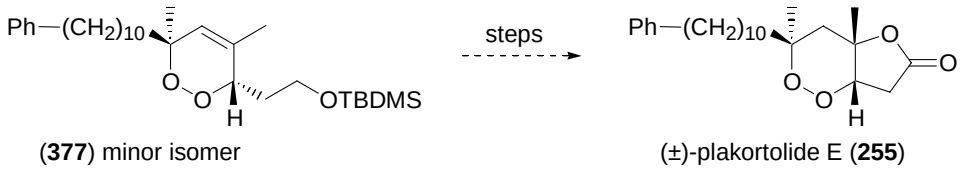
The absolute stereochemistry at the newly created C-3 and C-6 centers was deduced with the modified method of Mosher so that the diastereomeric peroxy esters have the absolute stereochemistries shown above. Additional comparisons suggest that the most likely stereochemistry of the natural $\Delta^{11,12}$ acid is (3*S*,6*R*,8*S*,10*R*).

The synthesis of 6-epiplakortolide E has also employed the singlet oxygen cycloaddition approach.[101] The key steps are summarized below.



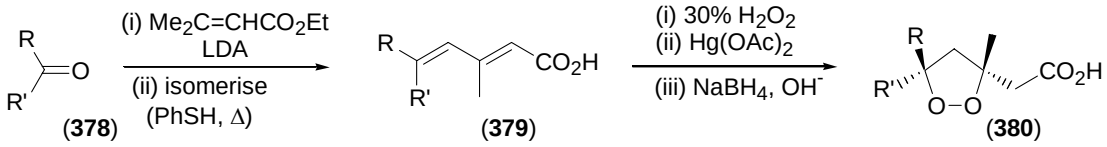

Scheme 49

Similar processing of the minor cycloadduct would afford plakortolide E (**255**) with the correct relative configuration, and other side-chain analogues of plakortolide would be accessible by this general approach.



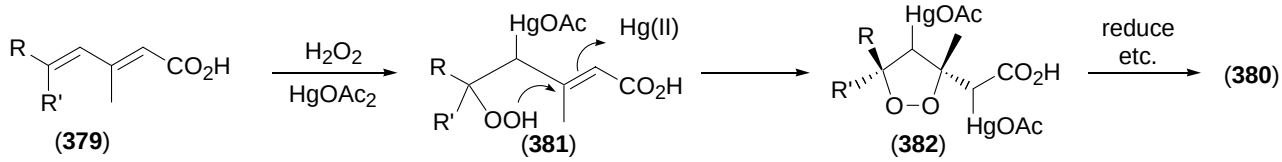

Scheme 50

Plakinic acids incorporate a 1,2-dioxolane moiety (in contrast to most other peroxy metabolites with 6-membered peroxide rings) and Bloodworth demonstrated the utility of peroxymercuration in constructing systems of this type,[102] with final borohydride removal of the mercury group. The optimized procedure is summarized below and was applied to acquire analogues of the plakinic acids, as well as the natural compound with R = Me and R' = $C_{15}H_{31}$.




Scheme 51

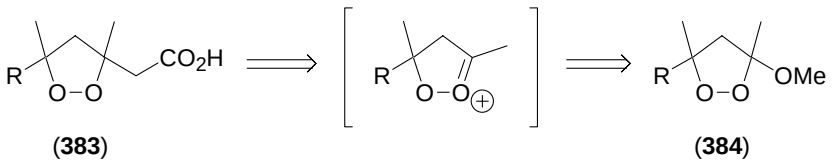
The process was envisaged to involve initial γ,δ-double bond addition, followed by regioselective intramolecular peroxymercuration.




Scheme 52

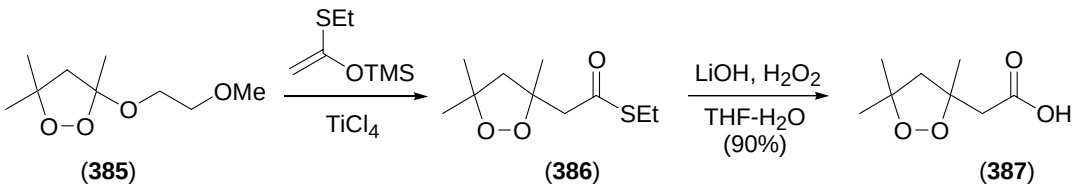
This approach may encounter difficulties in the presence of additional unsaturation as present for example, in plakinic acids A, C and D.

Dussault has also addressed the construction of the 1,2-dioxolane systems found in the plakinic acids,[103] and reported the addition of electron-rich alkenes to peroxycarbenium ions derived from 3-alkoxy-1,2-dioxolanes.

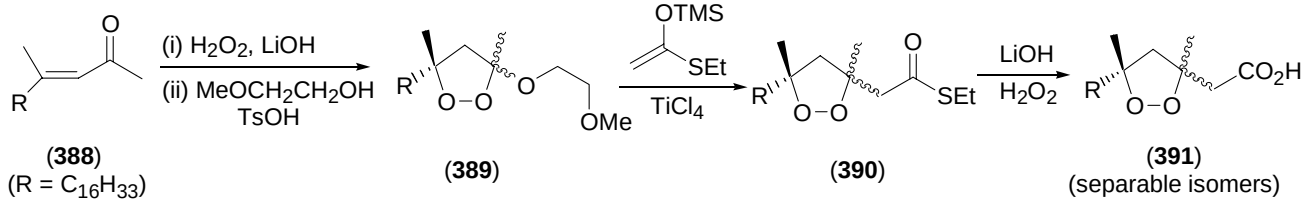



Scheme 53

Studies showed that either $SnCl_4$ or $TiCl_4$ and the 2-methoxy ethoxy leaving groups were useful combinations, and the silylenol ether of ethylthioacetate served as an acetate equivalent (Scheme 54). This methodology was then adapted to a synthesis of the 1,2-dioxolane system, related to the plakinic acids characterized from *P. halichondrioides* (Scheme 55).

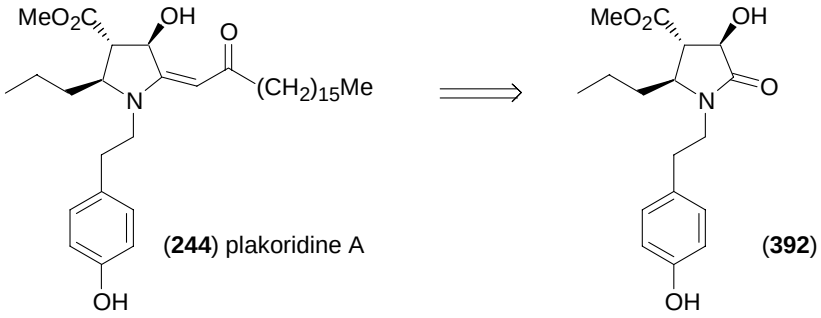



Scheme 54




Scheme 55

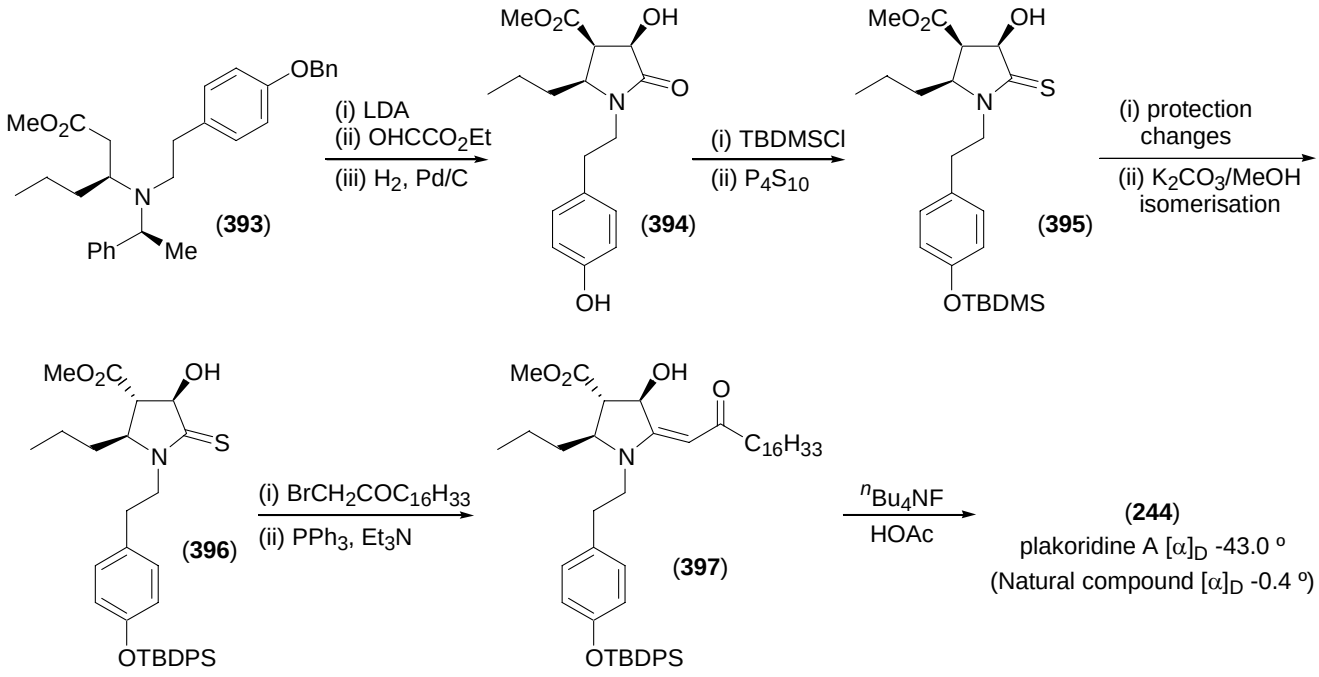
The plakoridine group of alkaloid metabolites has been the subject of synthetic interest and in 2000, Ma and Sun described the first synthesis of (2*S*,3*S*,4*R*)-plakoridine A from the lactam (**394**),[104] previously synthesized in racemic form by Stafford,[105] using nitrocycloaddition chemistry.




Scheme 56

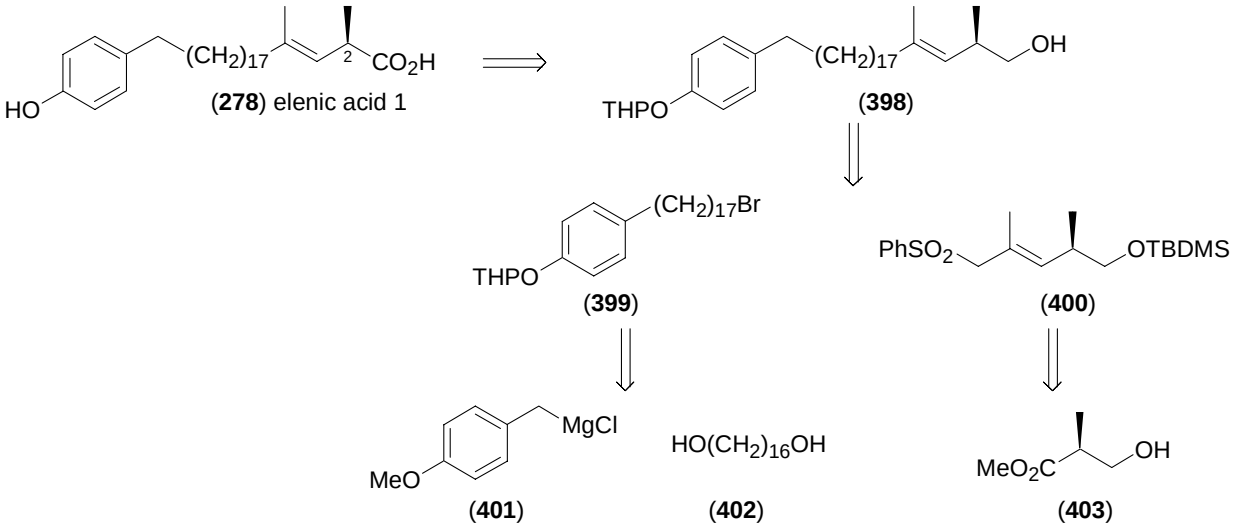
β-Amino ester [from (*S*)-α-methylbenzylamine] (from Michael addition of (*E*)-2-hexenoate) was transformed to the desired pyrrolidinone and then to the thiolactam which was isomerized at the ester-bearing center. *S*-Alkylation and subsequent Eschenmoser sulfide contraction worked well and deprotection afforded (2*S*,3*S*,4*R*)-plakoridine A.

The synthetic material had matching spectra with the natural product but a much larger optical rotation which surprisingly implies a racemic natural compound. This methodology appears capable of affording other polysubstituted pyrrolidines or piperidines.




Scheme 57

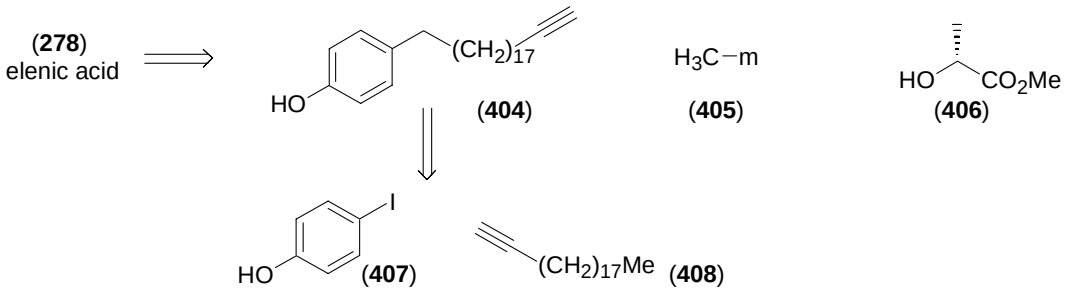
Elenic acid 1, an inhibitor of topisomerase II, isolated from a *Plakinastrella* sp.,[82] is unusual in that a β,γ-unsaturated acid is linked by a polymethylene chain to a phenol, with the sole stereogenic centre being at C-2 (methyl group). Mori described a synthesis guided by the retrosynthesis shown below.[106]




Scheme 58

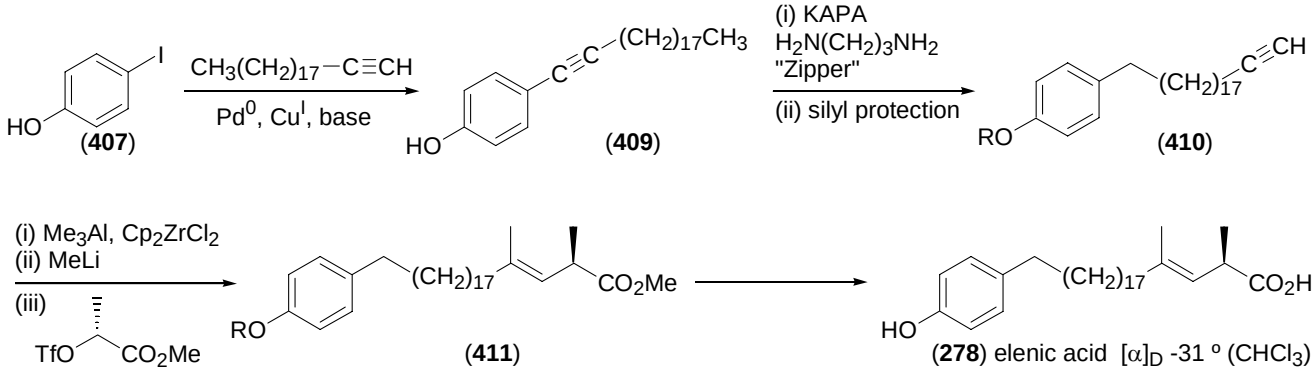
The synthesis based on this plan provided (*R*)-(–)-elenic acid 1, with $[\alpha]_D$ –30 º ($CHCl_3$) and estimated to be 87% ee (HPLC) and spectroscopically matching the natural product. ($[\alpha]_D$ –27.2 º).

Subsequently, both enantiomers of elenic acid were acquired by Hoye,[107] with lactate being the source of chirality, and involving a "one pot" conversion of an alkyne to an (*E*)-β,γ-unsaturated ester, with a stereocenter at the α-position.



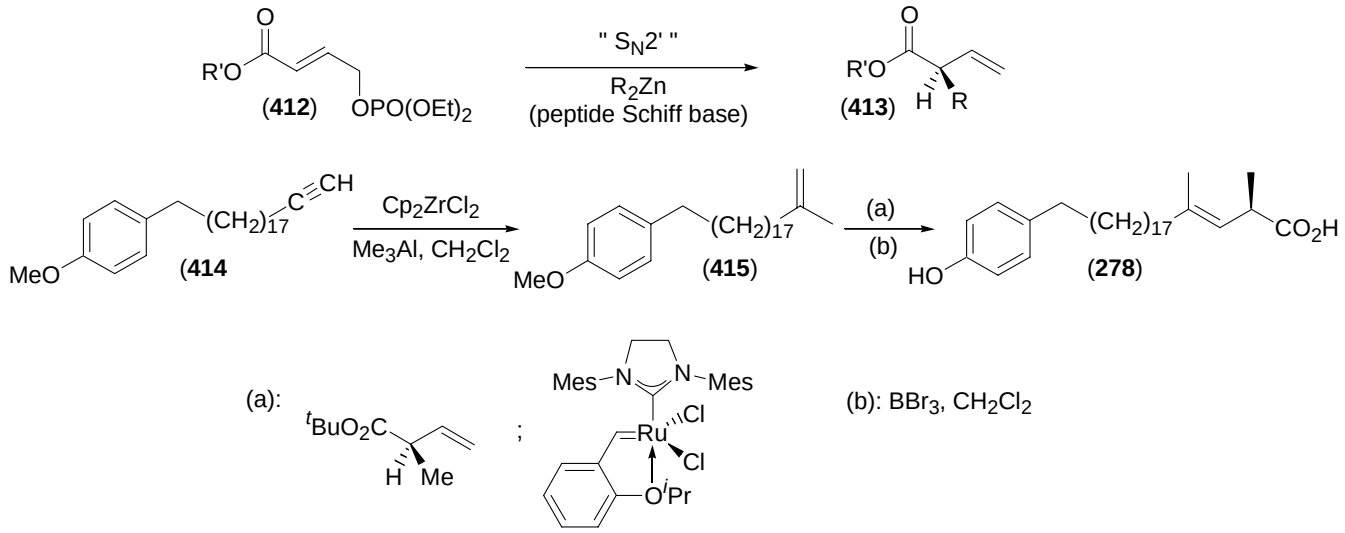

Scheme 59

The execution of this plan to yield (*R*)-(–)-elenic acid is summarized below. Similarly, (*S*)-(+)-elenic acid resulted from the use of the triflate from methyl (*S*)-(–)-lactate.




Scheme 60

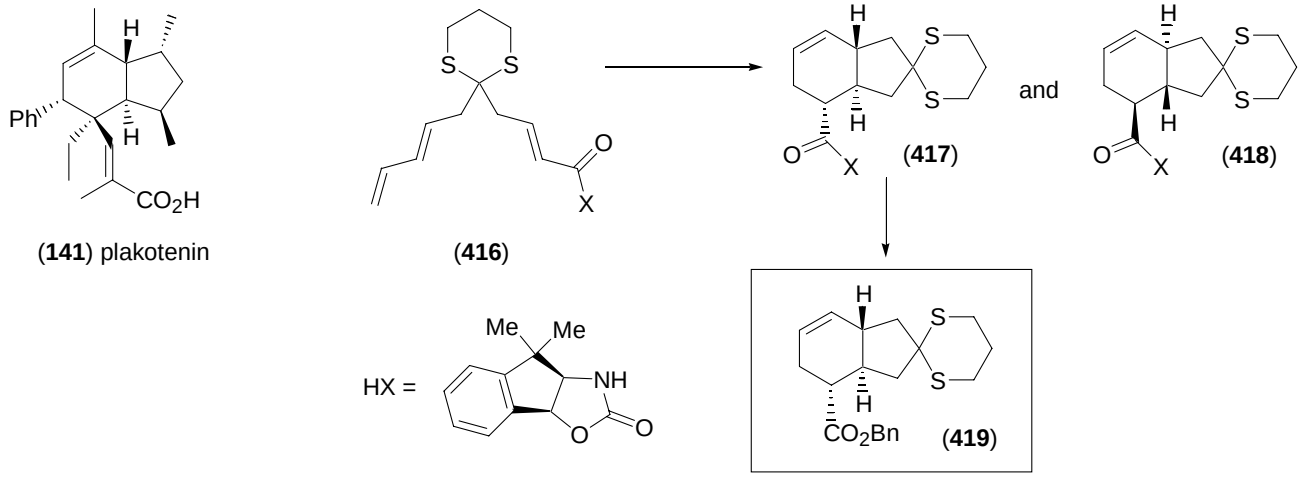
An efficient Cu-catalyzed enantioselective method for allylic alkylation of α,β-unsaturated esters with alkyl zinc reagents was adapted for the synthesis of (*R*)-(–)-elenic acid.[108] This reaction proceeds with high ee with a primary γ-phosphate leaving group.




Scheme 61

The sequence in (a) utilizes cross-metathesis between the aromatic alkene and the enantio-enriched β,γ-unsaturated ester. Finally deprotection affords (*R*)-(–)-elenic acid.

Plakotenin (**141**), incorporating a *trans*-hydrinane core, has attracted some synthetic effort. Diastereoselective asymmetric intramolecular Diels-Alder reactions of the type below have been performed following the suggestion that biosynthesis of plakotenin might involve [4+2] cycloaddition.[109]

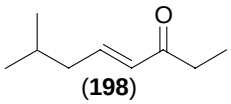



Scheme 62

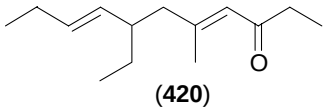
Best results were achieved with an oxazolidinone auxiliary, providing (**419**) (96% ee), a potentially useful intermediate towards plakotenin.

Synthesis of (*E*)-7-methyloct-4-en-3-one (**198**) (from *P. zygompha*) and 7-ethyl-5-methyl-4*E*,8*E*-undecadien-3-one (**420**) (from *P. halichondrioides*) have also been reported by straightforward methods,[110]

the former involving a Knoevenagel reaction with a β-keto acid, and the latter a Claisen rearrangement and standard manipulations.

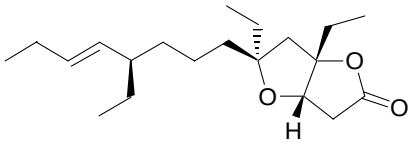


(**198**)
*P. zygompha*

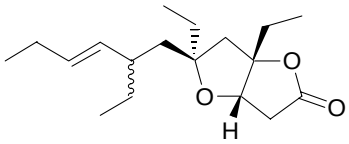


(**420**)
*P. halichondrioides*

More recent work has focused on the synthesis in the plakortone family of compounds, particularly plakortone D and plakortone E.[111] In the former, the relative stereochemistry was based on NMR spectral data but that at C-10 was not determined, nor was the absolute stereochemistry.

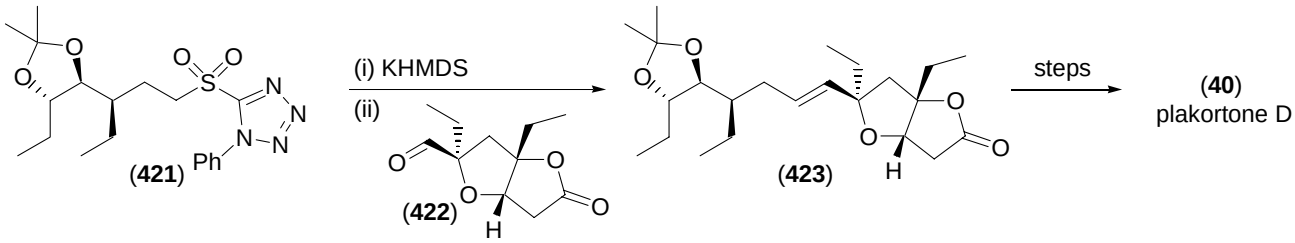


(**40**) plakortone D



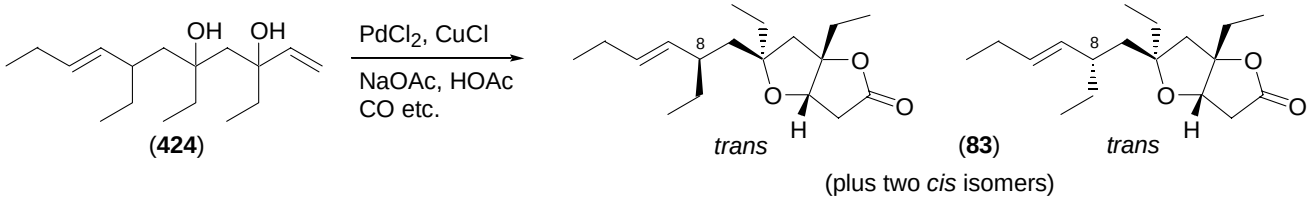
(**83**) plakortone E

A total synthesis of plakortone D, utilizing Sharpless asymmetric dihydroxylation methodology for chirality introduction, confirmed that plakortone D has the structure and absolute stereochemistry shown above. A key step involved a palladium (II)-mediated-hydroxy-cyclization-carbonylation-lactonization cascade. The lactone core and side chain were independently generated with known absolute stereochemistry, and then coupled with a sulfone anion.




Scheme 63

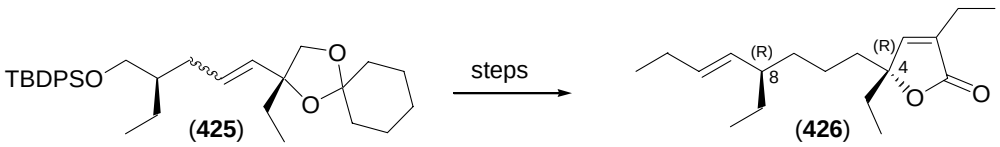
A similar approach, again using the Pd(II)-mediated cascade has operated efficiently with an enediol to provide four separable diastereomers of the bicyclic lactone assigned to plakortone E. All are *cis*-fused and one is identical with natural plakortone E, thus confirming its constitution and relative stereochemistry, about the lactone core.




Scheme 64

Plakortone E is one of the two *trans*-isomers above with the relative stereochemistry at C-8 relative to the lactone core, undetermined. An enantioselective synthesis of plakortone E is currently being developed, and is very likely to possess the (3*S*,4*S*,6*S*,8*R*) stereochemistry.

The total synthesis of plakortone G,[77] and thereby the determination of its absolute stereochemistry have been recently achieved. Keys steps for stereochemical enforcement were an Evans asymmetric alkylation, and Sharpless asymmetric dihydroxylation. The final α,β-unsaturated γ-lactone unit was delivered by an intramolecular aldolisation and elimination as summarized below. Plakortone G was shown to have the (4*R*,8*R*)-configuration.[112]




(**425**) (**426**)

Scheme 65

## SUMMARY

The structure, stereochemistry, synthesis and biological activities of metabolites generated by sponges of *Plakortis* sp. have been reviewed. These metabolites manifest a rich structural diversity and exciting range of biological activity, which together should maintain ongoing interest in this area of marine natural product chemistry.

## ACKNOWLEDGEMENTS

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## REFERENCES

1. M. D. Higgs and D. J. Faulkner, *J. Org. Chem.*, 1978, **43**, 3454.
2. B. N. Ravi, R. W. Armstrong, and D. J. Faulkner, *J. Org. Chem.*,1979, **44**, 3109.
3. D. B. Stierle and D. J. Faulkner, *J. Org. Chem.*, 1980, **45**, 3396.
4. D. B. Stierle and D. J. Faulkner, *J. Org. Chem.*, 1979, **44**, 964.
5. E. W. Schmidt and D. J. Faulkner, *Tetrahedron Lett.*, 1996, **37**, 6681.
6. A. D. Patil, A. J. Freyer, B. Carte, R. K. Johnson, and P. Lahouratate, *J. Nat. Prod.*, 1996, **59**, 219.
7. A. D. Patil, A. J. Freyer, M. F. Bean, B. K. Carte, J. W. Westley, R. K. Johnson, and P. Lahouratate, *Tetrahedron*, 1996, **52**, 377.
8. A. Rudi and Y. Kashman, *J. Nat. Prod.*, 1993, **56**, 1827.
9. D. E. Williams, T. M. Allen, R. Van Soest, H. W. Behirsch, and R. J. Andersen, *J. Nat. Prod.*, 2001, **64**, 281.
10. Y. Chen, P. J. McCarthy, D. H. Harmody, R. Schimoler-O'Rourke, K. Chilson, C. Selitrennikoff, S. A. Pomponi, and A. E. Wright, *J. Nat. Prod.*, 2002, **65**, 1509.
11. (a) M. d. S. Jiménez, S. P. Garzón, and A. D. Rodríguez, *J. Nat. Prod.*, 2003, **66**, 655; (b) M. d. S. Jiménez, S. P. Garzón, and A. D. Rodríguez, *J. Nat. Prod.*, 2003, **66**, 1404.
12. A. Rudi, R. Talpir, Y. Kashman, Y. Benayahu, and M. Schleyer, *J. Nat. Prod.*, 1993, **56**, 2178.
13. E. Quinoa, E. Kho, L. V. Manes, P. Crews, and G. J. Bakus *J. Org. Chem*. 1986, **51**, 4260.
14. A. Rudi, R. Afanii, L. G. Gravalos, M. Aknin, E. Gaydou, J. Vacelet, and Y. Kashman, *J. Nat. Prod.*, 2003, **66**, 682.
15. B. S. Davidson, *Tetrahedron Lett.*, 1991, **32**, 7167.

16. (a) A. Qureshi, J. Salvá, M. K. Harper, and D. J. Faulkner, *J. Nat. Prod.*, 1998, **61**, 1539; (b) A. Qureshi, J. Salvá, M. K. Harper, and D. J. Faulkner, *J. Nat. Prod.*, 2001, **64**, 553.
17. B. S. Davidson, *J. Org. Chem.*, 1991, **56**, 6722.
18. In this connection, see: V. Costantino, E. Fattorusso, M. Menna, and O. Taglialatela-Scafati, *Current Medicinal Chemistry*, 2004, **11**, 1671.
19. V. Costantino, E. Fattorusso, A. Mangoni, M. Di Rosa, and A. Ianaro, *J. Am. Chem. Soc.*, 1997, **119**, 12465.
20. (a) T. Natori, M. Morita, K. Akimoto, and Y. Koezuka, *Tetrahedron*, 1994, **50**, 2771; (b) K. Motoki, E. Kobayashi, T. Uchida, H. Fukushima, and Y. Koezuka, *Bioorg. Med. Chem. Lett.*, 1995, **5**, 705.
21. V. Costantino, E. Fattorusso, A. Mangoni, M. Di Rosa, and A. Ianaro, *Bioorg. Med. Chem. Lett.*, 1999, **9**, 271.
22. V. Costantino, E. Fattorusso, A. Mangoni, M. Di Rosa, and A. Ianaro, *Tetrahedron*, 2000, **56**, 1393.
23. V. Costantino, E. Fattorusso, C. Imperatore, and A. Mangoni, *Eur. J. Org. Chem.*, 2001, 4457.
24. V. Costantino, E. Fattorusso, C. Imperatore, and A. Mangoni, *J. Nat. Prod.*, 2002, **65**, 883.
25. V. Costantino, E. Fattorusso, C. Imperatore, and A: Mangoni, *Tetrahedron*, 2000, **56**, 3781.
26. V. Costantino, E. Fattorusso, C. Imperatore, and A. Mangoni, *Tetrahedron*, 2001, **57**, 4045.
27. F. Cafieri, E. Fattorusso, O. Taglialatela-Scafati, and A. Ianaro, *Tetrahedron*, 1999, **55**, 7045.
28. F. Cafieri, E. Fattorusso, O. Taglialatela-Scafati, M. Di Rosa, and A. Ianaro, *Tetrahedron*, 1999, **55**, 13831.
29. Plakortide H (**92**) was originally isolated as the corresponding methyl ester (**36**), see ref. 6, but only the relative stereochemistry around the peroxide ring was then determined.
30. E. Fattorusso, O. Taglialatela-Scafati, M. Di Rosa, and A. Ianaro, *Tetrahedron*, 2000, **56**, 7959.
31. E. Fattorusso, S. Parapini, C. Campagnuolo, N. Basilico, O. Taglialatela-Scafati, and D. Taramelli, *Journal of Antimicrobial Chemotherapy*, 2002, **50**, 883.
32. Plakortolide E (**97**) was originally reported as the corresponding free acid (**41**), see ref. 7. Plakortide M (**99**) was originally named plakortide I, but since this name already was given to another compound (**93**), see ref. 30, it was renamed, see ref. 77a-b.
33. C. Campagnuolo, E. Fattorusso, O. Taglialatela-Scafati, A. Ianaro, and B. Pisano, *Eur. J. Org. Chem.*, 2002, 61.
34. N. Matsumori, D. Kaneno, M. Murata, H. Nakamura, K. Tachibana, *J. Org. Chem.*, 1999, **64**, 866.
35. C. Campagnuolo, E. Fattorusso, and O. Taglialatela-Scafati, *Eur. J. Org. Chem.*, 2003, 284.
36. F. Borrelli, C. Campagnuolo, R. Capasso, E. Fattorusso, and O. Taglialatela-Scafati, *Eur. J. Org. Chem.*, 2004, 3227.
37. C. Campagnuolo, C. Fattorusso, E. Fattorusso, A. Ianaro, B. Pisano, and O. Taglialatela-Scafati, *Org. Lett.*, 2003, **5**, 673.
38. The name plakortic acid had already been claimed for another compound (**42**), see ref. 8.
39. Y.-C. Shen, V. S. C. Prakash, and Y.-H. Kuo, *J. Nat. Prod.*, 2001, **64**, 324.
40. S. P. Gunasekera, M. Gunasekera, G. P. Gunawardana, P. McCarthy, and N. Burres, *J. Nat. Prod.*, 1990, **53**, 669.
41. D. M. Kushlan and D. J. Faulkner, *J. Nat. Prod.*, 1991, **54**, 1451.
42. R. S. Compagnone, I. C. Piña, H. R. Rangel, F. Dagger, M. V. R. Reddy, and D. J. Faulkner, *Tetrahedron*, 1998, **54**, 3057.
43. X.-H. Huang, R. van Soest, M. Roberge, and R. J. Andersen, *Org. Lett.*, 2004, **6**, 75.
44. N. Takada, M. Watanabe, A. Yamada, K. Suenaga, K. Yamada, K. Ueda, and D. Uemura, *J. Nat. Prod.*, 2001, **64**, 356.
45. A. Qureshi, C. S. Stevenson, C. L. Albert, R. S: Jacobs, and D. J. Faulkner, *J. Nat. Prod.*, 1999, **62**, 1205.
46. B. Harrison and P. Crews, *J. Nat. Prod.*, 1998, **61**, 1033.
47. F. S. De Guzman and F. J. Schmitz, *J. Nat. Prod.*, 1990, **53**, 926.
48. S. Sakemi, T. Higa, U. Anthoni, and C. Christophersen, *Tetrahedron*, 1987, **43**, 263.
49. M. Miller and L. S. Hegedus, *J. Org. Chem.*, 1993, **58**, 6779.
50. T. Higa, C. Cristophersen, and S. Sakemi, U.S. Patent #4,731,377, March 15, 1988.
51. J. S. Sandler, P. L. Colin, J. N. A. Hooper, and D. J. Faulkner, *J. Nat. Prod.*, 2002, **65**, 1258.
52. G. R. Pettit, T. Nogawa, J. C. Knight, D. L. Doubek, and J. N. A. Hooper, *J. Nat. Prod.*, 2004, In press. Web Release Date: August 12, 2004.
53. A. Zampella, C. Giannini, C. Debitus, and M. V. D'Auria, *Tetrahedron*, 2001, **57**, 257.
54. D. J. Faulkner and B. N. Ravi, *Tetrahedron Lett.*, 1980, **21**, 23.
55. N. M. Targett and G. P. Schmal, Skidaway Inst. Oceanogr., Savannah, GA (1984), Gov. Rep. Announce. Index (U.S.) 1984, 84(21), 98.
56. D. W. Phillipson and K. L. Rinehart, *J. Am. Chem. Soc.*, 1983, **105**, 7735.
57. R. J. Wells, *Tetrahedron Lett.*, 1976, 2637.
58. T. Murayama, Y. Ohizumi, H. Nakamura, T. Sasaki, and J. Kobayashi, *Experimentia*, 1989, **45**, 898.
59. M. Kobayashi, K. Kondo, and I. Kitagawa, *Chem. Pharm, Bull.*, 1993, **41**, 1324.
60. A. Fontana, M. Ishibashi, and J. Kobayashi, *Tetrahedron*, 1998, **54**, 2041.
61. S. I. Toth and F. J. Schmitz, *J. Nat. Prod.*, 1994, **57**, 123.
62. W. D. Inman, M. O'Neill-Johnson, and P. Crews, *J. Am. Chem. Soc.*, 1990, **112**, 1.
63. R. R. West, C. L. Mayne, C. M. Ireland, L. S. Brinen, and J. Clardy, *Tetrahedron Lett.*, 1990, **31**, 3271.

64. C. J. Smith, D. A. Venables, C. Hopmann, C. E. Salomon, J. Jompa, A. Tahir, D. J. Faulkner, and C. M. Ireland, *J. Nat. Prod.*, 1997, **60**, 1048.
65. S. Tsukamoto, S. Takeuchi, M. Ishibashi, and J. Kobayashi, *J. Org. Chem.*, 1992, **57**, 5255.
66. J. Kobayashi, S. Tsukamoto, S. Takeuchi, and M. Ishibashi, *Tetrahedron*, 1993, **49**, 5955.
67. J. Kobayashi, S. Takeuchi, M. Ishibashi, H. Shigemori, and T. Sasaki, *Tetrahedron Lett.*, 1992, **33**, 2579.
68. M. Ishibashi, S. Takeuchi, and J. Kobayashi, *Tetrahedron Lett.*, 1993, **34**, 3749.
69. F. Saito, R. Takeuchi, T. Kamino, K. Kuramochi, F. Sugawara, K. Sakaguchi, and S. Kobayashi, *Bioorg. Med. Chem. Lett.*, 2004, **14**, 1975.
70. S. Takeuchi, M. Ishibashi, and J. Kobayashi, *J. Org. Chem.*, 1994, **59**, 3712.
71. S. Takeuchi, T. Kikuchi, S. Tsukamoto, M. Ishibashi, and J. Kobayashi, *Tetrahedron*, 1995, **51**, 5979.
72. A. Fontana, M. Ishibashi, H. Shigemori, and J. Kobayashi, *J. Nat. Prod.*, 1998, **61**, 1427.
73. M. Tsuda, T. Endo, M. Perpelescu, S. Yoshida, K. Watanabe, J. Fromont, Y. Mikami, and J. Kobayashi, *Tetrahedron*, 2003, **59**, 1137.
74. (a) T. Ichiba, P. Scheuer, and M. Kelly-Borges, *Tetrahedron*, 1995, **51**, 12195; (b) T. Ichiba, P. Scheuer, and M. Kelly-Borges, *Tetrahedron*, 1996, **52**, 14079.
75. M. Varoglu, B. M. Peters, and P. Crews, *J. Nat. Prod.*, 1995, **58**, 27.
76. J. C. Braekman, D. Daloze, S. De Groote, J. B. Fernandes, and R. W. N. Van Soest, *J. Nat. Prod.*, 1998, **61**, 1038.
77. (a) J.-F. Hu, H.-F. Gao, M. Kelly, and M. T. Hamann, *Tetrahedron*, 2001, **57**, 9379; (b) J.-F. Hu, H.-F. Gao, M. Kelly, and M. T. Hamann, *Tetrahedron*, 2002, **58**, 1233; (c) J. Gochfeld and M. T. Hamann, *J. Nat. Prod.*, 2001, **64**, 1477.
78. S. P. Gunasekera, R. A. Isbrucker, R. E: Longley, A. E. Wright, S. A. Pomponi, and J. K. Reed, *J. Nat. Prod.*, 2004, **67**, 110.
79. P. A. Horton, R. E. Longley, M. Kelly-Borges, O. J. McConnell, and L. M. Ballas, *J. Nat. Prod.*, 1994, **57**, 1374.
80. The name plakortolide F was claimed for another compound (**281**) at the same time, see ref. 84. The name plakortolide had been assigned to another compound (**65**), see ref. 15. This compound is unnamed in the original paper by Faulkner, see ref. 3.
81. T. L. Perry, A. Dickerson, A. A. Khan, R. K. Kondru, D. N. Beratan, P. Wipf, M. Kelly, and M. Hamann, *Tetrahedron*, 2001, **57**, 1483.
82. E. G. Juagdan, R. S. Kalidindi, P. J. Scheuer, and M. Kelly-Borges, *Tetrahedron Lett.*, 1995, **36**, 2905.
83. Y. Mizushina, C. Murakami, K. Otha, H. Takikawa, K. Mori, H. Yoshida, F. Sugawara, and K. Sakaguchi, *Biochemical Pharmacology*, 2002, **63**, 399.
84. Y. Chen, K. B. Killday, P. J. McCarthy, R. Schimoler, K. Chilson, C. Selitrennikoff, S. A. Pomponi, and A. E. Wright, *J. Nat. Prod.*, 2001, **64**, 262.
85. The name plakortolide F was claimed for another compound (**271**) at the same time, see ref. 81.
86. M. Takagi, H. Takikawa, and K. Mori, *Biosci. Biotechnol. Biochem.*, 2001, **65**, 2065.
87. K. Takeda, I. Nakayama, and E. Yoshii, *Synlett*, 1994, 178.
88. M. Asami, T. Ishizaki, and S. Inoue, *Tetrahedron Lett.*, 1995, **36**, 1893.
89. H. Miyaoka, T. Watanuki, Y. Saka, and Y. Yamada, *Tetrahedron*, 1995, **51**, 8749.
90. C. Kuhn, L. Skaltsounis, C. Monneret, and J.-C. Floret, *Eur. J. Org. Chem.*, 2003, 2585.
91. S. Al-Busafi, M. G. B. Drew, T. Sanders, and R. C. Whitehead, *Tetrahedron Lett.*, 1998, **39**, 1647.
92. S. Al-Busafi and R. C. Whitehead, *Tetrahedron Lett.*, 2000, **41**, 3467.
93. S. Al-Busafi, J. R. Doncaster, M. G. B: Drew, A. C. Regan, and R. C. Whitehead, *J. Chem. Soc., Perkin Trans. 1*, 2002, 476.
94. J. R. Doncaster, H. Ryan, and R. C. Whitehead, *Synlett*, 2003, 651.
95. K. C. Nicolaou, J. Li, and G. Zenke, *Helv. Chim. Acta*, 2000, **83**, 1977.
96. (a) M. Seki, A. Kayo, and K. Mori, *Tetrahedron Lett.*, 2001, **42**, 2357; (b) M. Seki and K. Mori, *Eur. J. Org. Chem.*, 2001, 3797.
97. (a) K. Mori, T. Tashiro, K. Akasaka, H. Ohrui, and E. Fattorusso, *Tetrahedron Lett.*, 2002, **43**, 3719; (b) T. Tashiro, K. Akasaka, H. Ohrui, E. Fattorusso, and K. Mori, *Eur. J. Org. Chem.*, 2002, 3659.
98. (a) B. B. Snider and Z. Shi, *J. Org. Chem.*, 1990, **55**, 5669; (b) B. B. Snider and Z. Shi, *J. Am. Chem. Soc.*, 1992, **114**, 1790.
99. (a) P. H. Dussault and K. R. Woller, *J. Am. Chem. Soc.*, 1997, **119**, 3824; (b) P. H. Dussault, C. T. Eary, and K. R. Woller, *J. Org. Chem.*, 1999, **64**, 1789.
100. G. Yao and K. Steliou, *Org. Lett.*, 2002, **4**, 485.
101. M. Jung, J. Ham, and J. Song, *Org. Lett.*, 2002, **4**, 2763.
102. A. J. Bloodworth, B. D. Bothwell, A. N. Collins, and N. L. Maidwell, *Tetrahedron Lett.*, 1996, **37**, 1885.
103. P. H. Dussault and X. Liu, *Org. Lett.*, 1999, **1**, 1391.
104. D. Ma and H. Sun, *Tetrahedron Lett.*, 2000, **41**, 1947.
105. J. A. Stafford, *Tetrahedron Lett.*, 1995, **36**, 681.
106. S. Takanashi, M. Takagi, H. Takikawa, and K. Mori, *J. Chem. Soc., Perkin Trans 1*, 1998, 1603.
107. R. C. Hoye, A. S. Baigorria, M. E. Danielson, A. A. Pragman, and H. A. Rajapakse, *J. Org. Chem.*, 1999, **64**, 2450.

108. K. E. Murphy and A. H. Hoveyda, *J. Am. Chem. Soc.*, 2003, **125**, 4690.
109. M. Ishizaki, Y. Hara, S. Kojima, and O. Hoshino, *Heterocycles*, 1999, **50**, 779.
110. (a) D. H. Grayson and M. R. J. Tuite, *J. Chem. Soc., Perkins Trans 1*, 1986, 2137; (b) O. P. Vig, R. Vig, D. M. Dua, and S. K. Puri, *Indian J. Chem.*, 1983, **22B**, 1126.
111. (a) P. Hayes and W. Kitching, *J. Am. Chem. Soc.*, 2002, **124**, 9718; (b) P. Hayes and W. Kitching, *Heterocycles*, 2004, **62**, 173; (c) P. Hayes, F. Rahm, S. Chow, and W. Kitching, Research in progress.
112. S. Kowashi, T. Ogamino, J. Kamei, Y. Ishikawa, and S. Nishiyama, *Tetrahedron Lett.*, 2004, **45**, 4393.