POLYKETIDE DERIVED METABOLITES FROM THE MARINE HYDROID
GARVEIA ANNULATA

by

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ABSTRACT

The marine hydroid Garveia annulata is a small, brightly colored coelenterate whose crude methanol extracts exhibit potent antibacterial and antifungal activity. The compounds responsible for this biological activity were purified and characterised as a series of related 1-[4H]-anthracenone derivatives. Twenty one metabolites have been isolated and their structures were elucidated by using a combination of spectral analysis, chemical interconversions, synthesis and single crystal X-ray diffraction analysis.

The structure of garveatin A (77), the major metabolite, was determined via a series of NMR experiments and by an X-ray diffraction analysis of its enol triacetate 87. Garveatins B (88), C (93) and D (96) share a common carbon skeleton with 77.

The garvin family represents a different polyketide folding pattern as seen in garvin A (124) and garvin B (126) which contain an n-propyl group and a delta lactone functionality, respectively. Both the garveatins and the garvins contain oxidized analogs in the form of 2-hydroxy derivatives, 9,10 quinones and C2,2' dimers. NMR analysis and optical rotation experiments indicate that the C2 position of the 2-hydroxy compounds is racemic.

Garvalones A (137) and B (141) represent the corresponding 2-(3-oxobutyl) derivatives of garvins A and B respectively. They
occur as pairs of C2 epimers. Their structures were confirmed by spectral comparison with 2-(3-oxobutyl) garveatin A (140) which was synthesised from 77.

Annulins A (144) and B (148) have degraded anthracene skeletons and they appear to be products of garveatin B metabolism.

All four families of G. annulata secondary metabolites appear to be produced by straightforward polyketide biogenesis. Different folding patterns of a putative nonaketide precursor account for all the structures elaborated. These polyketides represent the first examples of this type of metabolism in coelenterates.
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ABBREVIATIONS

CDCl₃ = Chloroform-d₁
CHCl₃ = Chloroform
CH₃CN = Acetonitrile
DMSO = Dimethylsulfoxide
EtOAc = Ethyl acetate
HPLC = High performance liquid chromatography
HRMS = High resolution mass spectrum
i = impurity peak
IR = Infra red
MeOH = Methanol
MS = (Low resolution) mass spectrum
Na₂SO₄ = Sodium sulfate (anhydrous)
¹H NMR = Proton nuclear magnetic resonance
¹³C NMR = Carbon -13 nuclear magnetic resonance
nOe = nuclear Overhauser enhancement
mp = melting point
RT = room temperature
S = solvent signal
SFORD = Single frequency off resonance decoupled
SCUBA = Self contained underwater breathing apparatus
TLC = Thin layer chromatography
TMS = Tetramethyl silane
UV = Ultra violet
w = water signal
"We dance round a ring and suppose,
But the secret sits in the middle and knows."

R. Frost (The Secret Sits)
I: INTRODUCTION.

A. MARINE NATURAL PRODUCTS CHEMISTRY.

Research in natural products chemistry (the study of secondary metabolites) has traditionally focused on terrestrial species, mainly from the plant and microbial kingdoms, as sources of compounds which exhibit interesting biological activity. Many of the pharmacologically active drugs in use today, such as morphine, atropine and streptomycin are natural products or semi-synthetic derivatives thereof.

Until recently, the vast number of organisms found in the oceans of the world have been largely untapped as a source of biologically active molecules. However, the great potential of the marine environment has been recognised for some time, as evidenced in the results of screening tests conducted in the early 1970's on over 1600 diverse marine species. The results showed that 9% contained compounds with antitumor activity, compared with 2-3% of terrestrial organisms.

A number of technological advances in the last 25 years have catalysed the growth of marine natural products chemistry, and attracted researchers into this area of study. The advent of SCUBA diving has greatly facilitated the collection of sub-tidal organisms, which were previously quite inaccessible compared to their terrestrial counterparts. Advances in the use of physical techniques such as NMR spectroscopy, mass spectrometry and x-ray diffraction analysis has enabled researchers to elucidate the
structures of compounds present in very small amounts, a situation often encountered with marine metabolites.

Screening large numbers of marine organisms has revealed some generalities about the distribution of biologically active substances\(^2\). Considerable interest has recently been focused on members of the more primitive marine phyla as sources of secondary metabolites. Creatures particularly rich in toxic chemicals include slow-moving, shell-less molluscs like sea-hares and nudibranchs, and soft-bodied sessile organisms like sponges, tunicates, soft-corals and gorgonians. Clues about the presence of bioactive compounds can be obtained by studying the ecology and behaviour of marine organisms. For example, in densely populated habitats such as coral reefs and tidepools, selection pressures have favored the evolution of chemical defenses. Many organisms can synthesize toxic substances or concentrate them from food sources in order to deter predators or keep competitors from approaching too closely. Thus, many families of marine invertebrates that outwardly appear to be totally defenseless actually have few predators.

The potential of the marine environment as a source of new pharmaceuticals is beginning to be realised as a number of compounds are currently undergoing clinical trials as antibiotic, antitumor, analgesic and anti-inflammatory agents\(^3,4\). The compound 1-B-D-arabino- furanosyl cytosine (ARA-C)\(^5\)(1) is used in cancer chemotherapy as an anti-leukemic drug. It is a derivative of spongouridine (ARA-U) (2) isolated from the sponge Cryptothetia crypta\(^6\) and more recently from the gorgonian
Eunicella cavolini.

The tunicates, comprising some 2000 species, are particularly rich in bioactive substances. Rinehart's group has isolated the cyclic peptide didemnin (3) from the Caribbean tunicate Tridemnum cyanophorum. Clinical tests in-vitro have shown that this molecule inhibits the growth of L1210 leukemia cells at a concentration of only 0.001 umole/ml.

The sessile, plant-like, colonial invertebrates from the phylum Bryozoa have yielded a number of interesting metabolites in recent years. Pettit's group from Arizona State have obtained a series of anti-tumor compounds named bryostatins from the bryozoan Bugula neritina, a species that forms moss-like colonies on ships hulls and other marine equipment. These compounds are strongly cytotoxic and anti-leukemic, and it has been suggested that the 20 membered lactone ring structure acts as an ionophore, making cell membranes porous to certain ions. Bryostatin 1 (4) is an example of this family of metabolites.

A series of four bipyroles, named tambjamines A-D (5-8) have been isolated from the bryozoan Sessibugula translucens by Faulkner's group and they show antimicrobial and cytotoxic activity. These compounds were previously found in nudibranchs of the Tambje genus.

The British Columbia bryozoans Phidolopora pacifica and Diaperoecia californica have yielded the purine alkaloids phidolopin (9) and desmethyl phidolopin (10), containing the rare naturally occurring nitro group. These compounds are responsible for much of the antifungal and antialgal activity associated with extracts of this organism.
$\begin{align*}
5 & \quad X = H \\
6 & \quad X = \text{Br} \\
7 & \quad X = H \\
8 & \quad X = \text{Br}
\end{align*}$
\[ R = \text{CH}_3 \]

\[ R = \text{H} \]
The above examples serve to illustrate that metabolites from marine sources exhibit a wide variety of biological activities. Usually, these compounds are assumed to act as chemical defenses for the host organisms, however, most of the evidence is circumstantial, due to the difficulties of testing the defensive hypothesis.

The literature on marine natural products chemistry has been extensively reviewed in a series of books and review articles by Scheuer and Faulkner, illustrating the rapid progress and diversification achieved in this field of research in the last twenty years.

This thesis will describe the isolation and structure elucidation of a group of novel secondary metabolites from the Pacific hydroid *Garveia annulata* which exhibit potent in-vitro antibacterial and antifungal activity. These compounds, which appear to be of polyketide origin, represent an interesting variation in coelenterate chemistry and this study will hopefully contribute to the growing body of research on the chemistry and ecological roles of bioactive metabolites from the ocean.
B. HYDROID ZOOLOGY AND CHEMISTRY

The marine hydroid *Garveia annulata* is a member of the phylum Coelenterata (or Cnidaria), containing about 9000 living species. In addition to hydroids, this phylum also includes jellyfishes, sea-anenomes and corals. Coelenterates show a number of distinguishing features\(^{17-20}\). They have a body wall consisting of two layers of cells. The outer layer is called the epidermis, and the inner layer, which forms the lining of the gut is called the gastrodermis. Between these two layers is a jelly-like substance known as the mesoglea. Coelenterates exhibit two different body forms, the medusa which is adapted to a pelagic existence, and the polyp which is adapted to an sessile benthic existence.

Most species feed on zooplankton and fine particulate matter. Prey is caught with the tentacles surrounding the oral cavity and immobilised by specialised stinging cells called nematocysts which are unique to this phylum. The mouth is the only opening into the gut cavity, and these animals lack organs or differentiated muscle cells.

There are three classes of coelenterates: the Hydrozoa, Scyphozoa and Anthozoa (Figure 1.). The Scyphozoa are composed mainly of the large jellyfishes, whereas the sea-anenomes and most hard corals belong to the Anthozoa. The class Hydrozoa is subdivided into two orders: the Siphonophora which contain free-swimming species such as the freshwater organism *Hydra*, and the
Figure 1. Phylogenetic classification of the marine hydroid *Garveia annulata*. 
Hydroidea, composed mainly of sessile colonial organisms known as hydroids. *G. annulata* belongs to the latter order.

The hydroids are generally small, relatively inconspicuous, plant-like organisms which are often dismissed as "sea-weed". They are wholly aquatic, being found in both fresh and salt water, and they exhibit a metagenic life-cycle composed of an asexual polypoid stage and a sexual medusa stage. A hydroid colony consists of a network of interconnected polyps or zooids which share a common gastrointestinal cavity. The three body layers in a hydroid colony, epidermis, mesoglea and gastrodermis are all continuous, making it difficult to say where one individual begins and another ends. The organism is attached to a rock or other substratum via a basal stem known as a hydrorhiza or stolon. Branches or pedicels extend from this structure which in turn give rise to bell-shaped hydranths. A single polyp consists of a hydranth and the part of the stalk between the hydranth and the point of origin of the preceding branch. The external surface of the colony is protected by a chitinous layer called the perisarc which is expanded into bell-shaped hydrothecae to accomodate the hydranths. Around the mouth of the hydranth are located a number of tentacles which contain nematocysts at the extremities. The hydranth captures, ingests and digests food, which then is transported to the common gastrovascular cavity.

Hydroids are polymorphic organisms that contain reproductive polyps called gonozoooids in addition to the hydranths (gastrozooids). These gonozoooids produce medusae by asexual
Figure 2. Life cycle of the colonial hydroid *Obelia*.\textsuperscript{17}
budding. The medusae eventually become detached and leave the colony. Some of the medusae produce eggs while others produce spermatozoa. Fertilised eggs give rise to ciliated larvae, called planulae, which become fixed to some object and form a new colony. This is the first stage of hydroid generation. As the young polyp grows it puts out branches that terminate in new polyps. Eventually, reproductive polyps appear and another generation of medusae is produced. Figure 2 illustrates the life-cycle of *Obelia*, a typical colonial hydroid.

*G. annulata*, which is found along the Pacific coast of North America from Sitka, Alaska to Santa Catalina Island, California, was identified by Nutting in 1901 from samples obtained by the Harriman expedition in Alaska (see Figure 3). It typically inhabits exposed rocky reefs at a depth of 2-120 metres and is most abundant in late winter and early spring. Colonies of *G. annulata*, which are 5-10 cm tall and consist of 20-30 polyps, are often found growing on or through sponges and algae. The most distinctive feature of this organism is its bright orange colour, making it quite conspicuous compared to most other hydroids. The oval-shaped gonophores are sessile medusoid structures which on a given colony are either all male or all female. Male medusoids shed their sperm to the sea and fertilise eggs from the female medusoids. These eggs are retained by the female gonophores and are eventually liberated as planula larvae, which can settle and form a new colony. Little else is known of the species. *G. annulata* gets its name from the numerous rings or annulations on the stems of the colonies.
Figure 3. *Garveia annulata*: (a) actual size, (b) section of stem showing hydranths, (c) gonophores.
Hydroids seem to have received scant attention from zoologists and chemists alike, possibly due to their small and inconspicuous nature and to the difficulties in collecting and identifying the different species. With the exception of this thesis, the limited number of hydroid chemical studies have all been carried out on Mediterranean species. In 1980, Cimino\textsuperscript{23} reported the isolation of the novel polyhydroxylated steroid \textsuperscript{11}, which is characterised by a C18 oxygen functionality, from the hydroid \textit{Eudendrium} sp. More recently, Fattorusso's group \textsuperscript{24,25} have isolated three steroids \textsuperscript{12-14} from \textit{Eudendrium glomerulatum}, all of which were hydroxylated at the 2, 3, and 18 positions.

\textit{E. glomerulatum} \textsuperscript{26} has also yielded four acyclic, polyhalogenated monoterpenes \textsuperscript{15-18}, which had been previously isolated from the red alga \textit{Plocamium cartilagineum} and the sea-hare \textit{Aplysia limacina}. 


C. CONDENSED POLYKETIDES FROM MARINE INVERTEBRATES

Introduction

All of the organic soluble metabolites discussed in this thesis appear to be derived from linear polyketide precursors which are subsequently modified by a variety of condensation, methylation, alkylation and oxidation reactions. In order to place the array of metabolites obtained from *G. annulata* in perspective, a review of polyketide-derived compounds from marine organisms is presented below. Although none of these metabolites were obtained from organisms belonging to the same phylum (Coelenterata) as *G. annulata*, many structural similarities were evident, and the literature reviewed provided many helpful insights into the structure elucidation of the *G. annulata* metabolites.

The marine environment has long been recognised as an abundant source of brightly coloured organisms, especially from the phyla Echinodermata and Coelenterata. The Echinoderms, many of which have brilliantly pigmented outer surfaces, have attracted considerable attention from chemists over the last 100 years due to their relative ease of collection. Echinoderms are divided into five distinct classes: *Crinoidea* (sea-lilies), *Asteroidea* (sea-stars), *Ophiuroidea* (brittle-stars), *Holothuroidea* (sea-cucumbers) and *Echinoidea* (sea-urchins)\(^\text{17}\). All of these classes have been shown to elaborate pigments of
polyketide origin but researchers in this area have concentrated on sea-urchins (Echinoidea) and the crinoids (Crinoidea).

**Echinoidal Pigments**

Sea-urchins are protected by numerous calcareous spines of varied hues which protrude from an oval shell or test. The spines and tests contain polyhydroxylated naphthoquinone pigments bound as salts that are released upon dissolution of the calcareous material with strong acid. The compounds isolated to date can be divided into two groups based on the presence of juglone (19) or naphthazarin (20) skeletons. A semi-trivial nomenclature based on these structures has been adopted.

The study of echinoidal pigments was initiated by Mc Munn in 1885 when he reported the presence of a red compound called "Echinochrome A" in the sea-urchin *Echinus esculentus*. The structure was shown to be 6-ethyl-2,3,7-trihydroxynaphthazarin (21) by Wallenfels in 1939, and verified by synthesis.

Prior to the 1960's, research in this area had been greatly hindered by inadequate methods for purifying these pigments, then known as "spinochromes", and by misleading combustion analyses, resulting in different structure proposals for the same compound by different groups. The advent of NMR spectroscopy and improved mass-spectral instruments, allowed groups led by Scheuer in Hawaii, Sutherland in Australia and Thomson in Scotland to clarify the situation by correctly assigning the structures of the spinochromes A-E (22-26). These pigments are widely distributed in sea-urchins, and the synthesis of several spinochromes has been reported by Scheuer and Thomson.
OH
O

24  COCH₃

OH

25 OH

26 OH

OH

OH

21

Cpd.  R₁   R₂   R₃
22  COCH₃  H   OH
23  OH     H   H
24  COCH₃  OH  OH
25  OH     H   OH
26  OH     OH  OH
By 1970, about twenty pigments containing substituted juglone and naphthazarin skeletons had been identified. The range of substituents included acetyl, ethyl, hydroxyl and methoxyl groups. The co-occurrence of methoxylated naphthazarins (27,28) in *Diadema antillarum* Phillips raised the possibility that some of the isolated naphthoquinones are artifacts formed by hydrolysis of methylated natural products in the 6N HCl solution used in the extraction procedure. It is quite possible that a 2,3-dimethyl precursor of 27 or 28 could be monodemethylated during the HCl digestion process, considering how rapidly this type of reaction occurs in refluxing ethanol-HCl.

An interesting feature of the naphthazarin-type compounds is the possibility of tautomerism due to the presence of hydroxyl groups at the 5- and 8- positions. NMR studies and transacylation reactions have been utilised by Moore and Scheuer to decide which is the predominant tautomer in solution. When naphthazarin is monosubstituted with methoxy, hydroxy, ethyl or acetoxyl groups, the structure was shown to be 29 in chloroform solution. This assignment was based on the observation that the C3 proton resonates further upfield than the C6 and C7 aromatic protons in model juglones and naphthoquinones. Also, when R=Et, the C3 proton is present as a sharp triplet due to allylic coupling with the methylene protons of the side-chain, indicating the presence of a fixed double bond in the quinone ring rather than a delocalised bond.

In contrast, 2-acetylnaphthazarin has structure 30, indicating an 'aromatic attraction' of the acetyl group. Thus in
\[ R = \text{OH, OCH}_3, \text{OAc or Et} \]

27 \( R_1 = \text{CH}_3 \), \( R_2 = \text{H} \)

28 \( R_1 = \text{H} \), \( R_2 = \text{CH}_3 \)

29

30

31
disubstituted naphthazarins containing one acetyl group, the nature of the predominant tautomer is determined by the degree of 'quinoidal attraction' or 'aromatic attraction' of the other substituent.

Mass spectrometry has also proved quite useful in structure determination of these molecules. Djerassi and Scheuer have proposed a set of empirical rules for analysing the mass-spectral fragmentation of naphthoquinones. Their rules are based on results from a large number of natural and synthetic compounds. As would be expected of brightly coloured pigments, UV-visible spectroscopy is a valuable tool in the gross structural analysis of the naphthoquinones and in the determination of their substitution patterns.

A number of more unusual compounds, in addition to the simple juglone and naphthazarin derivatives outlined above, have been isolated from echinoids. The pigment 2-methyl-8-hydroxy-2H-pyrano[3,2g]naphthazarin (31) isolated from *Echinothrix diadema* represented the first sea-urchin compound with a side-chain greater than two carbon atoms.

Thomson's group has reported the binaphthoquinone ethylidene -3,3-bis(2,6,7-trihydroxynaphthazarin) (32) and its anhydro derivative 33 from the spines of *Spatangus purpureus*. Compound 32 did not exhibit a molecular ion in the mass spectrum. Instead, the spectrum contained a base peak at m/z 238 and the fragmentation pattern was essentially the same as that displayed by 34, a compound present in minor amounts in the same extract. Methylation of 32 with diazomethane yielded the hexamethyl ether.
32 \ R = H \quad 35 \ R = CH_3
which gave a weak molecular ion at m/z 586 indicative of a dimeric structure. Structure 32 was confirmed by condensing trihydroxynaphthazarin (34) with acetaldehyde to give a biquinone identical by TLC, UV and IR with 32.

The instability of the biquinones 32 and 35 on acid-treated silica gel is noteworthy since one of the breakdown products is compound 34. However, the authors argue that 34 is a genuine natural product since treatment of 32 with cold concentrated HCl for 12 hours did not promote decomposition. Dehydration of 32 with hot sulphuric acid yielded an anhydro compound identical with 33.

The role of the naphthoquinone pigments in the metabolism and ecology of the echinoids remains an open question. Reports of the absence of particular compounds from different specimens of the same species indicate that a central metabolic role is unlikely. Vevers has proposed that these compounds may function as algistats, which is in keeping with the defensive role of a number of natural products from various other marine invertebrates such as sponges, corals and nudibranchs.

Inspection of the structural formulae of the naphthoquinone compounds suggests a polyketide origin and support for this biosynthetic pathway has been provided by Lederer's group who have shown that [2-14C] labelled acetate is incorporated into 6-ethyl-2,3,7-trihydroxynaphthazarin (21) by the sea-urchin *Arabacia pustulosa*. It is noteworthy that several plant products are closely related to the echinoidal pigments, and 2,7-dihydroxynaphthazarin (36), a constituent of several sea-urchins, has also

23
been found in the fungus *Helicobasidium mompa* Tanaka.49

**Crinoidal Pigments**

The crinoids are the most ancient class of the phylum Echinodermata with an extensive fossil record consisting mainly of stalked crinoids (sea-lilies), some of which still exist. The majority of the living species are comprised of about 550 free-swimming comulatids, also known as feather-stars.

In contrast to the echinoids, the crinoids generally do not contain naphthazarin or juglone derivatives, but instead produce compounds based on anthraquinone (37) and naphthopyrone (38) structures. These compounds are easily extracted from the organisms with acetone or other organic solvents.

Studies of the chemistry of the highly coloured crinoids started with the work of Moseley on board the Challenger in the East Indies in 187452. Moseley described spectroscopically the pigments "purple pentacrinin", "red pentacrinin" and "antedonin" from a number of deep-sea species. Subsequently in 1890, Mc Munn examined the extract of a crinoid which is now believed to be *Ptilometra australis* Wilton53.

As was the case for the echinoidal pigments, the molecular structure of these compounds remained a mystery until the 1960's when Sutherland's group in Queensland, Australia published the structures of three anthraquinones isolated from the bright red crinoid *Comatula pectinata* 54. These are called rhodocomatulin 6-methyl- (39) and 6,8-dimethyl ethers (40) and rubrocomatulin 7-methyl ether (41).

Evidence for the butyryl side-chain in the rhodocomatulins
was provided by the formation of 1,3,6,8-tetrahydroxyanthraquinone (42) and butyric acid on treatment of 39 or 40 with refluxing hydrobromic-acetic acid. This reaction is apparently facilitated by steric hindrance between the butyryl group and the C10 carbonyl, forcing the side-chain out of the plane of the ring. It is interesting to note the similarity of the lichen pigment solorinic acid (43) with the above compounds\(^55\). In 1972, Thomson reported the structure of rhodolamprometrin (44) from specimens of Lamprometra klunzingeri collected in the Red Sea\(^56\). It differs from 39 only in the replacement of the butyryl side-chain with an acetyl group and the absence of the C6 methoxyl group. Compound 41, which contains an extra hydroxyl group, has a UV-visible spectrum typical of 1,4,5-trihydroxyquinones and its infrared spectrum indicates that both carbonyls are hydrogen-bonded. The three compounds 39-41 have also been isolated as their water-soluble C2-sulfate esters\(^57,58\). Sutherland later isolated a group of three pigments from the crinoid Ptilometra australis Wilton and named them rhodoptilometrin (45), isorhodoptilometrin (46) and rhodoptilometric acid (47)\(^59\).

These compounds differ from the Comatula pigments in that they contain side-chains in the beta positions rather than in the alpha positions. NMR evidence and the observed optical activity of 45 indicated the presence of a \(\text{CHOHCH}_2\text{CH}_3\) group. This assignment was further validated by the identification of propionic acid as one of the products of Kuhn-Roth degradation of 45. The isomeric compound isorhodoptilometrin (46) differs from 45 only in the nature of the hydroxypropyl side-chain.
45 \( R_1 = H \quad R_2 = (S)\text{-CHOHEt} \)
46 \( R_1 = H \quad R_2 = \text{CH}_2\text{CHOHCH}_3 \)
47 \( R_1 = \text{COOH} \quad R_2 = \text{CH}_2\text{CH}_2\text{CH}_3 \)
48
49 \( R \)
50 \( R \)
51 \( R \)
Isorhodoptilometrin 6-methyl ether (48) has previously been described as nalgiovensin, isolated from the mould *Penicillium nalgiovensis* Laxa. It is interesting to note that O-methylation characteristic of the *Comatula* compounds does not seem to occur in the *Ptilometra* pigments.

Studies by Scheuer's group on the Pacific crinoid *Comanthus bennetti* provide further examples (49-51) of anthraquinones alkylated at the C3 position. Compound 49 was previously known as a degradation product of the corresponding trimethyl ether, ptilometric acid, while rhodoptilometrin (45) could be oxidised to yield 50.

As well as the anthraquinones, crinoids also contain a variety of interesting substituted naphthapyrones. Sutherland's group isolated the linear naphthapyrone comantherin sulfate (52) from the species *Comanthera perplexa*. The sulfate ester functionality makes this compound water-soluble, and acid hydrolysis yields the hydrophobic comantherin (53). A second compound, neocomantherin (54), which differs from 53 only in its C3 substituent, was isolated from the acid hydrolysate. It is presumably present in the crinoid as the sulfate ester 55. Methylation of 53 yielded a compound identical with the known rubrofusarin dimethyl ether (56). The 5-dimethyl derivative of neocomantherin (54) was subsequently isolated from *Comantheria briarius* Bell in 1980.

Green specimens of the crinoid *Comanthus parvicurrus timorensis* yielded the closely related angular naphthopyrones 57-59, present as sulfate esters. It was noticed that if the
52. $X = \text{SO}_3^-$  $R = \text{CH}_3$
53. $X = \text{H}$  $R = \text{CH}_3$
54. $X = \text{H}$  $R = \text{CH}_3$
55. $X = \text{SO}_3^-$  $R = \text{CH}_3$
56. $X = \text{CH}_3$  $R = \text{CH}_3$
57. $R = \text{H}$  $R_1 = \text{H}$
58. $R = \text{OCH}_3$  $R_1 = \text{H}$
59. $R = \text{OCH}_3$  $R_1 = \text{CH}_3$
crinoids were not transferred directly from the sea to acetone but were allowed to autolyse, the three corresponding sulfate-free compounds were detected, presumably formed by the action of a sulfatase. Sulfation of these phenols with sulfamic acid in pyridine yielded products of $R_f$ identical with those observed for the water-soluble compounds 57-59.

The list of polyketide pigments from crinoids has recently been extended to include three 10,10'-bianthrones 60-62 derived from the known compounds 44 and 46. These bianthrones typically contain a principal ion corresponding to the anthrone formed by cleavage of the central bond and transfer of a H radical. The relative sterochemistry at the 10, 10' bond was ascertained to be as shown in 60 or its enantiomer, as opposed to the meso form, by comparison with the model compound emodin bianthrone 63. Natural 60 is optically active, indicating that the organism selectively produces one enantiomer. Minor amounts of the meso form were also detected in the extracts, possibly as an artifact. The relative stereochemistry of 61 and 62 has not yet been ascertained.

A minor component from the same crinoid was identified as the bianthraquinone 64 which is quite similar to the known bianthrone skyrin (65). The authors believe that 64 may be formed by aerial oxidation of 61 in the acetone extract, since there is a precedent for this type of reaction in bianthrones.

Similarly, the phenanthroperylenequinone 66 (also from the crinoid Lampometra palmata gyges) may also be an artifact, but the presence of compound 67 in the absence of bianthrones and biquinones in Himerometra robustipinna seems to indicate that the
60  \( R = \text{Pr} \quad R_1 = \text{Pr} \)
61  \( R = \text{Pr} \quad R_1 = \text{CHOHEt} \)
62  \( R = \text{CHOHEt} \quad R_1 = \text{CHOHEt} \)
63  \( R = \text{CH}_3 \quad R_1 = \text{CH}_3 \)
64  \( R = \text{Pr} \quad R_1 = \text{CHOHEt} \)
65  \( R = \text{CH}_3 \quad R_1 = \text{CH}_3 \)
crinoids are capable of synthesising these compounds.

It is interesting to note that a series of hydroxylated phenanthroperylenequinones (68) have been found in the fossilised remains of a stalked crinoid Apiocrinus (Millericrinus) by Blumer\textsuperscript{67}. These compounds, known as fringelites, may be genuine natural products or alternatively might be derived by geochemical modification of the corresponding bianthrones.

Attempts to correlate the presence of particular pigments with the taxonomy of the crinoid species studied so far has led to confusing results. Sutherland\textsuperscript{65} has postulated that the 'non-chemotaxonomic' distribution of polyketides in comatulid crinoids is due to the potential availability of a whole range of polyketides to all or many species, so that each species can adjust to its particular ecological environment by elaborating a different selection of compounds.

Experiments carried out by Sutherland's group\textsuperscript{57} indicate that the sulfated polyketides might provide crinoids with a chemical defense mechanism since they have been shown to have an inhibitory effect on feeding by fish. Surveys of the stomach contents of fish collected from areas where crinoids are known to be present revealed that crinoid fragments were typically absent, supporting the theory that these compounds render the crinoids unpalatable to predators.

With regard to the source of polyketide pigments in crinoidal tissue, it is unlikely that they are obtained from their diet since the two species Ptilometra australis and Comatula cratera have been found in the same habitat but contain
66. \( R = \text{Pr} \quad R_1 = \text{CHOHEt} \)

67. \( R_1 = \text{CH}_3 \quad R_1 = \text{CH}_3 \)

68. \( R = \text{H or OH} \)
completely different types of anthraquinones. In the absence of evidence of symbiotic organisms it is reasonable to assume that the crinoidal polyketides are of endogenous origin.

The similarity of crinoidal anthraquinones with compounds isolated from plants and lichens indicate that polyketide condensation patterns similar to those observed in the biosyntheses of plant anthraquinones may also occur in crinoids\(^6^8\). As Sutherland\(^5^9\) remarked: "Crinoids, which are so plant-like in external form have some synthetic capabilities which are typically manifest in plants rather than in animals".

**Miscellaneous**

The other four classes of the phylum Echinodermata have yielded pigments similar to those described above from echinoderms. For example, a monomethyl ether of 36 has been isolated from the holothuroid *Polycheria rufescens* Brandt\(^5^0\), while Singh\(^5^1\) and co-workers have isolated two dimethyl ethers of 26 from the asteroid *Acanthaster planci* Linn., the compounds 22 and 36 from an unidentified *Antedon* crinoid and several naphthoquinones including 21 and 22 from the ophiuroids *Ophiocoma erinaceus* Mill and Trosch and *O.insularia* Lyman.

Although the echinoderms have been the most prolific source of polyketide-derived metabolites to date, a number of other marine organisms contain examples of these compounds. The sea-worm *Halla parthenopeia* was found by Italian researchers\(^6^9\) to contain 7-hydroxy-8-methoxy-6-methyl-1,2-anthraquinone (69) known as hallochrome, which is responsible for the bright red colour of the epidermal cells of this species. Cimino has recently isolated
the corresponding hydroquinone 1,2,7-trihydroxy-8-methoxy-6-methyl anthracene (70) from the butanol extracts of the same organism, and has proposed that this compound is the biogenetic precursor of hallochrome 70.

Evidence that marine microorganisms also produce aromatic compounds of polyketide origin was provided by a Japanese group 71,72 in 1975. In the course of screening for antibiotics produced by actinomycetes from marine environments, a Chainia species was isolated from a sea mud and was shown to contain the benz(a)-anthraquinone 71 which was responsible for the antibiotic activity. This compound was very unstable to heat and light, being converted to the linear naphthacenequinone 72.
69

70

71

72
Plate 1. *Garveia annulata* (Nutting).
INTRODUCTION

The choice of a suitable marine organism for a natural products study is dictated by a number of factors, the most important being sufficient availability, interesting biological activity of its extracts and uniqueness with respect to previous studies. The hydroid *Garveia annulata* fulfills these criteria as outlined below.

*G. annulata* was collected by hand using SCUBA during the winter and early spring months on exposed rocky reefs, at depths of 2 to 20 metres, in the Deer group of islands in Barkley Sound, Vancouver Island, B.C. Upon immersion in methanol, the organism immediately imparts its bright orange coloration to the solvent, and in large quantities gives a dense brownish solution. Typical collections have yielded 12-16g of methanol-soluble material from 400g dry weight of hydroid, making it an extremely rich source of secondary metabolites relative to other cold-water organisms studied in this laboratory.

The first collections of *G. annulata* were made in 1983. Biological screening conducted on the crude methanol extract indicated the presence of substantial *in-vitro* antibacterial and antifungal activity. Therefore, this organism represented an obvious target for isolation and structure elucidation of the compound(s) responsible for the observed biological activity. In addition, the extreme paucity of data on natural-products from hydroids (only one novel metabolite from a hydroid had ever been
reported at that stage\textsuperscript{23}, increased the probability that \textit{G. annulata} extracts might well contain distinctively different chemistry from that of previously studied invertebrate species.

Examination of the extracts of \textit{G. annulata} has revealed the presence of four families of polyketide-derived metabolites. The purification and structure elucidation of each family will be discussed separately. They are as follows:

A: The garveatins.
B: The garvins.
C: The garvalones.
D: The annulins.

A. THE GARVEATINS

(i) Garveatin A

Preliminary investigations of the crude methanol extract of \textit{G. annulata} by silica-gel thin layer chromatography (TLC) using 1:50:50 acetic acid/ethyl acetate/hexane as an eluant (this solvent system will be referred to as the "standard TLC system" in the remainder of this thesis) indicated that the major component of the mixture was a yellow-orange compound with an \( R_f \) of 0.37. This major band had a dark yellow long-wave UV fluorescence. When acetic acid was omitted from the solvent system, this component showed considerable streaking on silica-gel, giving a bright yellow long-wave UV fluorescence. These observations indicated the presence of an acidic molecule containing a highly conjugated chromophore. The purification scheme used to purify this compound, later named garveatin A\textsuperscript{73},

39
is outlined below.

Freshly collected *G. annulata* was immersed in methanol in 4-litre plastic jars and stored at room temperature for 3-7 days. The densely pigmented methanol extract, typically about 8 litres, was filtered through Celite and evaporated *in vacuo* to give an aqueous suspension (400ml). This aqueous phase was successively extracted with hexane(3x400ml), methylene chloride(3x400ml) and ethyl acetate(2x400ml).

The initial fractionation involved vacuum-filtration chromatography using a 3.5 cm thick silica pad in a sintered-glass funnel (10cm dia.) attached to a filter-flask. This method was found to be preferable to the more conventional flash column setup, since the larger diameter of the funnel permitted higher concentrations of crude extract, and the shorter "column" length minimized irreversible adsorption to the silica gel. Stepwise elution of the methylene chloride phase of the hydroid extract(4g) from the above-mentioned suction-flash column, using ethyl acetate/hexane mixtures, yielded a fraction (1.5g) eluting with 100% ethyl acetate. This fraction was further subjected to Sephadex LH-20 chromatography using 90% MeOH/CH₂Cl₂ as the eluant. A bright yellow-orange band, which was strongly retarded and eluted as the final component of the mixture, proved to be almost pure garveatin A. Final purification of garveatin A was obtained by trituration with 80% CHCl₃/hexane and crystallization from acetone to give orange needles (300mg) (m.p.236-240°C).

HRMS analysis of garveatin A established the molecular formula as C₂₀H₂₀O₅ (M⁺ m/z 340.1317 obs., 340.1311 calc'd.). The intense molecular ion suggested an aromatic compound, and this
evidence was supported by its UV spectrum (λ_max 232, 282, 323(sh), 432nm in MeOH) which was typical of a polycyclic aromatic chromophore. The ^1H NMR spectrum (CDCl_3) contained aromatic resonances at 7.10 (brs,1H) and 7.15ppm (s,1H) whose upfield shift (relative to benzene) suggested that they were ortho or para to a phenol group. Additional evidence for the presence of phenol functionalities was provided by proton singlets at 10.56 and 17.34 ppm, their downfield chemical shifts indicating a strong H-bonding effect. Observation of a green color on spraying with FeCl_3 solution also pointed to the presence of a phenol group chelated to a carbonyl.

Analysis of the ^13C NMR spectrum of garveatin A (75 MHz, acetone- d_6) indicated the presence of ten aromatic resonances at 106.0(s), 110.1(d), 113.9(s), 118.3(d), 120.6(s), 136.9(s), 138.5(s), 149.1(s), 161.6(s), and 170.5 ppm(s), the two most downfield resonances being appropriate for carbons bearing phenols. Additional ^13C NMR signals at 204.1(s) and 32.7 ppm(q), in conjunction with a ^1H NMR singlet at 2.68 ppm(3H), suggested the presence of an aromatic acetyl group. ^13C NMR data for the model compound o-hydroxyacetophenone (73) gives a value of 204.4 ppm for the carbonyl carbon in CDCl_3 solution, while the ^1H nmr shift of the aromatic acetyl group in the juglone derivative (74) is exactly the same as in garveatin A.

A broad singlet at 2.40 ppm (3H) in the ^1H NMR spectrum was assigned to an aromatic methyl group exhibiting long-range coupling to the aromatic proton at 7.10 ppm (brs, 1H). This coupling pattern was confirmed by performing a decoupling.
experiment on the trimethyl derivative of garveatin A, where irradiation of the broad singlet at 7.41ppm produced a sharpening of the methyl singlet at 2.38ppm. In addition, a difference NOE experiment on the same derivative in which the broad aromatic singlet at 7.41 ppm was irradiated produced an enhancement in the intensities of both the aromatic proton at 7.65ppm and the aromatic methyl at 2.38ppm. These experiments established that the two aromatic protons were peri with respect to each other and that the aromatic methyl group was ortho to one of the aromatic protons. The above data led us to propose partial structure for garveatin A, which is analogous to the known compound musizin.

Musizin is a constituent of wood from a species of an African tree and contains an IR band at 1630 cm⁻¹, due to the strongly chelated carbonyl group. This compares favorably to the carbonyl stretching frequency of 1610 cm⁻¹ for garveatin A. In addition, the UV spectrum (light petroleum) of musizin contains maxima at 219, 266 and 402 nm, which is quite similar to the spectrum of garveatin A (MeOH) in acid solution (max 238, 253, 298, 402 nm).

The remaining part of the molecule has the formula C₇H₁₀O₂, and nine of the hydrogen atoms can be accounted for by singlets at 1.63 (6H) and 1.98ppm(3H) in the ¹H NMR spectrum. The former resonance was assigned to a gem-dimethyl and this was supported by ¹³C NMR signals at 28.9 (q, 2C) and 40.8ppm (s). The downfield position of the methyl group at 1.98ppm suggested that it was attached to an sp² carbon, either an olefin or an enol group,
although its $^{13}\text{C}$ NMR shift (7.1ppm) was quite shielded.

Consideration of the remaining three $^{13}\text{C}$ NMR resonances at 198.6 (s), 103.3(s) and 175.9 ppm(s) in garveatin A led to the proposal of a keto-enol system containing a methyl group on the central carbon atom. In order to satisfy the molecular formula, the keto-enol and gem-dimethyl functionalities must be incorporated into a six-membered ring fused to the naphthalene moiety to give structure 77 for garveatin A.

Garveatin A bears a close resemblance to ferruginin A (83), a pigment isolated from the berries of a group of tropical trees belonging to the genus *Vismia* that grow in Central and South America. The $^1\text{H}$ NMR spectrum of ferruginin A (acetone $d_6$) contains phenol resonances at 10.35 (s) and 17.55ppm(s), which compare closely to those of garveatin A (10.56 and 17.34 ppm). Also, there are two aromatic protons at 7.26 and 7.02 ppm, the latter resonance showing long-range coupling to the aromatic methyl group at 2.40 ppm, as observed with garveatin A. The enolised beta-diketone system is also present, however, the C2 position is unsubstituted and a gem-diprenyl group, instead of a gem-dimethyl group, exists at C4. Comparison of the spectral data of various methylated and acetylated derivatives of garveatin A with the corresponding analogs of ferruginin A provided further proof for the proposed structure (see Table 2).

Treatment of garveatin A with diazomethane produced a mono-methyl derivative 78, identified by a molecular ion at 354 daltons in the mass spectrum, and a methyl singlet (3H) at 4.04 ppm in the $^1\text{H}$ NMR spectrum. This compound was much more soluble in CDCl$_3$ than garveatin A and was also more stable in solution.
<table>
<thead>
<tr>
<th>Number</th>
<th>Chemical Structure</th>
<th>Specificity</th>
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<tbody>
<tr>
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<td></td>
</tr>
<tr>
<td>78</td>
<td>R = CH₃ R₁, R₂ = H</td>
<td></td>
</tr>
<tr>
<td>79</td>
<td>R, R₁, R₂ = CH₃</td>
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<td>80</td>
<td>R, R₂ = CH₃ R₁ = H</td>
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</tr>
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<td>81</td>
<td>R, R₁, R₂ = Ac</td>
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</tr>
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<td>82</td>
<td>R, R₂ = Ac R₁ = H</td>
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</tr>
<tr>
<td>83</td>
<td>R, R₁, R₂ = H</td>
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<td>R = CH₃ R₁, R₂ = H</td>
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</tr>
<tr>
<td>86</td>
<td>R, R₁ = CH₃ R₂ = H</td>
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Table 1: $^{13}$C NMR data for Garveatin A (acetone $d_6$) and Ferruginin A$^{79}$ (dioxane $d_8$). Chemical shifts in ppm from TMS.

<table>
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<tr>
<th>Carbon#</th>
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<th>Ferruginin A (83)</th>
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<td>1</td>
<td>198.64</td>
<td>192.6</td>
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<tr>
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Table 2: $^1$H NMR (CDCl$_3$) data for derivatives of Garveatin A and Ferruginin A$^{79}$ Chemical shifts in ppm from TMS.

<table>
<thead>
<tr>
<th>H on C#</th>
<th>77</th>
<th>78</th>
<th>79</th>
<th>80</th>
<th>83</th>
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<td>7.13</td>
<td>7.65</td>
<td>7.20</td>
<td>7.1</td>
<td>7.01</td>
<td>7.09</td>
<td>7.06</td>
</tr>
<tr>
<td>8OR</td>
<td>10.56</td>
<td>10.40</td>
<td>3.86*</td>
<td>3.91</td>
<td>10.1</td>
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</tr>
<tr>
<td>9OR</td>
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<td>17.25</td>
<td>3.97*</td>
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<td>16.5</td>
<td>16.85</td>
<td>15.48</td>
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</tr>
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</table>

* indicates assignments may be reversed.
The presence of two chelated OH protons at 10.40 and 17.25 ppm indicated that the C8 and C9 OH groups were unsubstituted, and this was supported by the fact that the aromatic proton shifts (7.01 and 7.13 ppm) were almost identical to those of garveatin A (7.02 and 7.15 ppm). Thus, methylation had occurred at the C3 position, and this was confirmed by an NOE experiment where irradiation of the gem-dimethyl group produced an enhancement of the C3 methoxy (4.04 ppm) and the C10 aromatic (7.13 ppm) protons. The $^1$H NMR and UV data is in excellent agreement with that of the methylated ferruginin A derivative 84 prepared by diazomethane treatment of 83 by Marini-Bettolo and co-workers.

Assignment of the $^{13}$C NMR spectrum of garveatin A was assisted by carrying out a long-range HETCOR (heteroscalar correlated spectroscopy) 2-D NMR experiment on a 400 mg sample. The HETCOR experiment establishes direct connectivities between two different bonded nuclei (in this case $^1$H and $^{13}$C) and therefore allows one type of nucleus (e.g. $^{13}$C) to be assigned from the known assignment of the other type. The pulse sequence used to produce the HETCOR spectrum (see Figure 4a) produces a transfer of polarization from the protons to the carbon nuclei and therefore leads to a four-fold enhancement in sensitivity in the $^{13}$C channel. Coupled nuclei yield signals with the coordinates (H), (C) in the 2-dimensional contour plot. Using appropriate delay times D3 and D4, the experiment can be optimised to detect long-range $^2J_{CH}$ and $^3J_{CH}$ couplings while suppressing the much larger $^1J_{CH}$ coupling. This approach was
Figure 4. Long-range HETCOR spectrum of 77 in acetone d$_6$
Figure 4a. Pulse Sequence for Long-range HETCOR Experiment on garveatin A (77).

\[
\begin{array}{c}
\text{\small $^1H$} \\
\begin{array}{c}
\text{\small 90°} \\
\text{\small D2} \\
\text{\small D3} \\
\text{\small 90°} \\
\text{\small DECOUPLE} \\
\end{array}
\end{array}
\begin{array}{c}
\text{\small $^1H$} \\
\begin{array}{c}
\text{\small 180°} \\
\text{\small D4} \\
\end{array}
\end{array}
\begin{array}{c}
\text{\small Status} \\
\text{\small A} \\
\text{\small B} \\
\text{\small C} \\
\end{array}
\]

INSTRUMENT: Varian XL-300.

PARAMETERS: 500 mg in 10 mm tube (acetone d$_6$).

$^{13}$C sweep width: 16,000 Hz. $^1$H NMR sweep width: 2250 Hz.

Delay time: 2 sec. Acquisition time: 64 msec. Number of increments: 128. 144 transients per increment. Number of points in t$_2$: 2048. Number of points in t$_1$: 512. J1XH set at 130 Hz. JNXH set at 10 Hz. Gives: D3 = 0.05 sec, D4 = 0.033 sec. Total acquisition time: 11 hours.
adopted for the HETCOR experiment on 77 since several of the aromatic carbons were not directly bonded to protons and therefore would not give correlations if a one-bond experiment was employed.

With the Varian XL-300 software, one enters a value of JNXH which corresponds to the long-range coupling constant that one wishes to observe, and the D3 (1/2J) and D4 (1/3J) values are then calculated automatically. A value of 10 Hz was used in this experiment even though $^{3}J_{CH}$ couplings involving aromatic hydrogens are usually 6-8 Hz\(^{82}\). Acquisition of the data took 11 hours and the resultant spectrum is shown in Figure 4, represented as a contour plot. It can be seen that both H5 and H10 protons transfer magnetisation to the $^{13}$C resonance at 113.9 ppm, identifying it as C8a. There is also a correlation between H10 (6.97 ppm) and the resonance at 106.0 ppm, assigned to C9a. Three-bond correlations between H5 (6.87 ppm) and C14, C10 and C7 can also be identified. Two-bond connectivities between C2-H11 and C4a-H12, H13 are present since $^{2}J_{CH}$ (typically 1-4Hz) is in the order of $^{3}J_{CH}$. It is somewhat discouraging to find a number of spurious peaks in the spectrum, especially in the upper right-hand region. Also, no correlation between H10 and C5 is observed which is surprising since these two nuclei should couple with a J value of about 7 Hz. These imperfections may be a consequence of the fact that compromise values of $^{1}J_{CH}$ and $^{3}J_{CH}$ were used to calculate the acquisition parameters, since the molecule contains both aromatic and aliphatic substructures. Ideally, it may be necessary to use a different set of parameters to optimise polarisation transfer through the different types of bonds in the
molecule.

The preferential methylation of garveatin A at the C3 position with diazomethane indicates that the C3 OH group is more nucleophilic or perhaps more acidic than the C8 and C9 OH groups. Evidence for the existence of an enolate anion at C3 is supported by the hypsochromic UV shift of garveatin A in acid (no shift was observed in base) and the absence of a C3 OH proton resonance in the \(^1\)H NMR spectrum. In addition, the compound streaks considerably when analysed by TLC in neutral solvents, but migrates as a tight spot on addition of 1% acetic acid, indicating a negatively charged species.

Methylation of garveatin A with dimethyl sulfate and \(K_2CO_3\) in refluxing acetone yielded the trimethyl derivative \(80\) as well as smaller amounts of the 3,8-dimethyl derivative \(79\). Assignment of a methoxy group at the C8 rather than the C9 position of the latter compound is based on the fact that the broad C5 aromatic proton is shifted farther downfield than the the C10 singlet on methylation. Also, comparison with the corresponding dimethyl analog of ferruginin A \(85\) substantiates this assignment.

Treatment of garveatin A with pyridine and acetic anhydride at room temperature overnight formed the triacetate \(81\) and lesser amounts of the 3,8-diacetate \(82\), analogous to the dimethyl sulfate methylation reaction. Three singlets in the \(^1\)H NMR spectrum (80 MHz, CDCl\(_3\)) of \(81\) at 2.35, 2.54 and 2.55 ppm were assigned to the acetate methyl groups, the most shielded resonance being appropriate for the C3 position. The aromatic protons were shifted downfield to 7.59 (H5) and 7.84 ppm (H10) as
a consequence of the deshielding effect of acetylation of the \textit{para} groups. The mass spectrum of \textit{81} shows a molecular ion at m/z 466 with successive loss of three 42 dalton fragments corresponding to the three acetate groups. Loss of 42 mass units is a very facile cleavage for aromatic and enol acetates and can be envisaged as a Mc Lafferty rearrangement resulting in loss of ketene and keto-enol tautomerisation of the resulting ketone to give the phenol (Scheme 1).

As was the case with the dimethyl derivative \textit{79} of garveatin A, the diacetyl derivative \textit{82} is acetylated at the C8 position, causing substantial deshielding of the C5 proton.

Garveatin A triacetate (\textit{81}), on slow evaporation in 1:1 CHCl$_3$/hexane at 4°C crystallised as yellow needles. However, X-ray diffraction analysis of one of the crystals which was performed by He Cun-heng and Jon Clardy of Cornell University revealed the presence of the isomeric structure (\textit{87}), which contains an enol acetate functionality instead of an aromatic acetyl group, and a phenolic OH group at C9. Subsequent TLC examination of the sample of crystals used in the X-ray analysis indicated the presence of a second compound, which gave a dark spot when exposed to I$_2$ vapour. Silica-gel chromatography was used to purify this compound, present in a 1:3 ratio with the triacetate. The $^1$H NMR spectrum of the minor compound contained two doublets at 5.00 and 5.38 ppm (1H each) with 2 Hz coupling, indicative of an olefinic methylene group. There was also a phenolic proton resonance at 15.33 ppm and the aromatic C10 proton (7.24 ppm) was shielded relative to the corresponding signal in the spectrum of the triacetate (\textit{81}), proving that the
Scheme 1.
C9 OH was not acetylated. The mass spectrum gave a molecular ion at 466 daltons, and was similar in many respects to that of the triacetate 81. Therefore, this minor compound corresponded to structure 87 obtained by X-ray analysis, and it evidently was formed by rearrangement of 81 during the crystallisation process.

In order to investigate the rearrangement reaction more fully, a sample of pure triacetate 81 was heated at 60°C in benzene containing a crystal of para-toluenesulfonic acid. Quantitative conversion to the enol-acetate isomer 87 occurred after 12 hours, indicating that the latter is the thermodynamically more stable structure. The computer-generated perspective drawing of 87 (Figure 5) indicates that both the C8 and C15 O-acetates are rotated roughly perpendicular to the plane of the aromatic nucleus to avoid serious steric repulsions. Triacetate 81 would be expected to give rise to strong steric repulsion between the C8 and C9 O-acetate groups and this strain could be relieved to some extent on rearrangement. It is possible that a trace of acid in the CHCl₃ used in the crystallisation of 81 catalysed the rearrangement in the sample submitted for X-ray diffraction analysis.

(ii) Garveatin B

The fraction (500 mg) obtained by elution of the suction-flash column with 50% ethyl acetate/hexane was subjected to LH-20 chromatography in 90% MeOH/CH₂Cl₂, followed by preparative TLC in 50% ethyl acetate/hexane to give a partially purified orange component with an Rf of 0.48 in the standard TLC system. Further
Figure 5. Computer generated X-Ray structure of the enol triacetate of garveatin A (87).
purification of this component on HPLC (normal-phase, ethyl acetate/hexane gradient) gave pure garveatin B (88) (15mg) as an orange yellow oil.

The $^1$H NMR spectrum (80 MHz, CDCl$_3$) of garveatin B$^{83}$ revealed that it was very closely related to garveatin A. Resonances at 1.62 (s, 6H), 1.99 (s, 3H), 2.46 (brs, 3H), 7.04 (brs, 1H), and 7.12 (s, 1H) were virtually identical to the corresponding resonances for the protons at C12 and 13, C11, C14, C5 and C10 respectively in garveatin A. The appearance of additional resonances at 1.18 (t, J=7Hz, 3H), and 2.80 ppm (q, J=7Hz, 2H) combined with the absence of an acetyl methyl resonance around 2.70 ppm in the spectrum of 88 indicated that garveatin B contained an ethyl side-chain at C7 instead of an acetyl group. A molecular formula of C$_{20}$H$_{22}$O$_4$, established by HRMS was consistent with this assignment and the UV (in MeOH) maxima of 240, 260, 317 and 417nm were quite similar to those of garveatin A.

An interesting feature of the $^1$H NMR spectrum of garveatin B in CDCl$_3$ solution is the presence of the diketo tautomer (90) in approximately 1:3 ratio with the keto-enol form. As a consequence, the phenolic protons appear as doubled peaks at 9.90, 10.25 ppm (1:3) and 16.23, 17.15 ppm (1:3), and there are weak resonances at 1.50(d, J=7Hz) and 3.98 ppm(q, J=7Hz) due to the protons at C11 and C2 respectively in the diketo tautomer. The upfield shift of the phenolic protons in the diketo form relative to the keto-enol form suggests a weaker H-bonding interaction with the Cl carbonyl group. This is likely due to
88  \( R = H \)
89  \( R = CH_3 \)
non-planarity of the Cl oxygen with the aromatic nucleus, or to a decrease in the electron density of the Cl oxygen in the diketo form due to the absence of resonance form 92 as indicated on page 57. The keto-enol tautomerism observed in the spectrum of garveatin B is quite similar to that reported for ferruginin A (83) in CDCl₃ solution 79, where it was observed that the proportion of the diketo form increased with increasing temperature. In addition, only the keto-enol form was present in acetone d₆ solution, as was the case for garveatin B.

Treatment of garveatin B with diazomethane yielded 3-methyl garveatin B (89) as the major product. In this compound, the keto-enol tautomer is "frozen" as a consequence of methylation of the C3 OH group, precluding any contribution from a diketo form. As a result, the ¹H NMR spectrum of 89 does not show any splitting of the resonances corresponding to the phenol protons.

(iii) Garveatin C

TLC analysis of the flash fraction eluting with 20% ethyl acetate/hexane (140 mg) indicated the presence of a component which had a white fluorescence under long-wave UV. LH-20 chromatography in 90% MeOH/CH₂Cl₂, followed by preparative TLC in 40% ethyl acetate/hexane gave pure garveatin C(93) (20 mg) which was responsible for the UV fluorescence. Garveatin C is more non-polar than garveatins A and B (Rf 0.54 in standard TLC system) and gave orange crystals (mp 125 C) on slow evaporation of a hexane solution at 4°C.
\[ R = H \quad R_1 = \text{CH}_3 \]

94 \[ R = \text{Ac} \quad R_1 = \text{CH}_3 \]

95 \[ R, R_1 = \text{CH}_3 \]

96 \[ R, R_1 = \text{H} \]

97 \[ R, R_1 = \text{H} \]

98 \[ R, R_1 = \text{Ac} \]

99 \[ R = \text{Ac} \quad R_1 = \text{H} \]
Comparison of the $^1$H NMR spectrum (80 MHz, CDCl$_3$) of 22 with that of garveatin A indicated many similarities, and resonances at 2.38 (brs, 3H), 2.62(s,3H), 1.58(s,6H), 7.19(s,1H) and 7.35(brs,1H) could be assigned to an aromatic methyl at C6, an aromatic acetyl at C7, a gem-dimethyl at C4 and aromatic protons at C10 and C5, by analogy. The remaining resonances were assigned to another gem-dimethyl group (1.49 (s,6H) ppm), an aromatic methyl ether (3.92, s,3H) and a phenolic proton (14.45 s,1H). The downfield shift of the C5 proton in 22 relative to its position in the spectrum of garveatin A (7.35 vs. 7.10 ppm) indicated that the aromatic methyl ether was at the C8 position. This follows from the deshielding influence of a methoxy group relative to a hydroxyl group on ortho and para positions in an aromatic nucleus. Consideration of the biogenesis of garveatin C indicated that the second gem-dimethyl group was at the C2 position and hypsochromic shifts in all the UV peaks relative to those in 22 was consistent with a less extensively conjugated chromophore.

The $^{13}$C NMR spectrum (see Table 4) of 22 contains three ketone carbonyl resonances at 211.6, 205.8 and 204.9 ppm and two quaternary sp$^3$ carbons at 48.1 and 55.2 ppm which support the proposed structure for the alicyclic ring. The most deshielded resonance at 211.6 ppm is appropriate for the unconjugated ketone at C3.

Acetylation of garveatin C (acetic anhydride/pyridine) gave a quantitative yield of the monoacetate (23). The chemical shift of the C2 gem-dimethyl (1.35 ppm) is 0.14 ppm upfield from that
in the $^1$H NMR spectrum of the parent compound, which suggests that introduction of an acetoxy group at the C9 position alters the anisotropic effect of the neighboring carbonyl group, resulting in a shielding of the C2 gem-dimethyl protons.

Final proof of the structure of 22 was achieved by converting both garveatins A and C to the dimethoxy derivative 25. Treatment of 22 with either diazomethane or dimethyl sulfate failed to yield any of the desired product, but methylation with methyl iodide and $K_2CO_3$ in refluxing acetone gave a quantitative yield of 25. Similarly, methylation of garveatin A with methyl iodide gave 25 as the major product. This reaction involves C-methylation at the C2 position of the diketo tautomer of 22, as well as O-methylation at the C8 and C9 positions.

(iv) Garveatin D

The flash fraction eluting with 100% ethyl acetate was further purified on LH-20 ($90\%$ MeOH/CH$_2$Cl$_2$) resulting in a fraction containing a mixture of minor metabolites, which were subjected to preparative TLC (a: 10% ethyl acetate/CHCl$_3$, b: 25% ethyl acetate/hexane) to give garveatin D (26) (8 mg) as a yellow oil. The mass spectrum of 26 showed a parent ion at m/z 354 daltons, appropriate for a molecular formula of $C_{21}H_{20}O_5$. The $^1$H NMR spectrum (270 MHz, CDCl$_3$) of 26 showed a striking resemblance to that of 22, the only noticeable difference being the absence of a methoxy group around 3.9 ppm, an upfield shift in the position of the C5 proton (7.15 vs. 7.35 ppm) reflecting the lack of methylation at C8, and the presence of two phenolic resonances.
Table 3: $^1$H NMR data (CDCl$_3$) for Garveatins A-D

Chemical shifts in ppm from TMS.

<table>
<thead>
<tr>
<th>H on C#</th>
<th>A (77)</th>
<th>B (88)</th>
<th>C (93)</th>
<th>D (96)</th>
</tr>
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<tbody>
<tr>
<td>5</td>
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<td>7.04</td>
<td>7.35</td>
<td>7.05</td>
</tr>
<tr>
<td>8OR</td>
<td>10.65</td>
<td>10.25</td>
<td>3.92</td>
<td>10.20</td>
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<tr>
<td>9OH</td>
<td>17.34</td>
<td>17.15</td>
<td>14.45</td>
<td>16.15</td>
</tr>
<tr>
<td>10</td>
<td>7.15</td>
<td>7.12</td>
<td>7.15</td>
<td>7.12</td>
</tr>
<tr>
<td>11</td>
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<td>2.46</td>
<td>2.38</td>
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<td>-</td>
<td>2.80 q</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>16</td>
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<td>1.18 t</td>
<td>2.62</td>
<td>2.63</td>
</tr>
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</table>

All resonances are singlets unless otherwise specified.
Table 4: $^{13}$C NMR data for Garveatin compounds.

Chemical shifts in ppm from TMS.

<table>
<thead>
<tr>
<th>C#</th>
<th>$A(77)^a$</th>
<th>C(93)$^b$</th>
<th>$D(96)^b$</th>
<th>A quinone(105)$^b$</th>
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<td>205.4*</td>
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<td>107.1</td>
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<td>139.4#</td>
<td>131.9$^&amp;$</td>
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<td>24.6</td>
<td>24.6</td>
<td>8.1</td>
</tr>
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<td>28.5</td>
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<td>32.3</td>
<td>31.9</td>
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<td>64.1</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

*,#,,$,\& indicates assignments may be reversed.
a: run in acetone D$_6$. b: run in CDCl$_3$. 

63
(10.20, 16.15 ppm) instead of just one as in 93. The structure 96 was proposed for garveatin D on the basis of the above data.

(v) 2-Hydroxygarveatin A.

Examination of the most polar flash chromatography fractions (eluted with 100% ethyl acetate and 20% MeOH/ethyl acetate) revealed the presence of two major metabolites which were yellow-orange in colour and had $R_f$'s of 0.1 and 0.28 in the standard TLC system.

LH-20 chromatography (90% MeOH/CH$_2$Cl$_2$) of this material produced a fraction containing approximately 100mg of an orange solid, a portion of which was fractionated on a reversed phase preparative TLC plate (80% MeOH/H$_2$O) to give 10 mg of pure 2-hydroxy garveatin A (97). Compound 97 was poorly soluble in organic solvents, and it was therefore characterised as its triacetate 98, prepared by treatment with acetic anhydride and pyridine. Triacetate 98 was a colourless solid with a molecular formula of C$_{26}$H$_{26}$O$_9$ (HRMS m/z observed 482.1567, req'd 482.1577). Comparison of its $^1$H NMR spectrum (80 MHz, CDCl$_3$) to that of triacetyl garveatin A (81) indicated the presence of identical aromatic rings in both molecules. However, aliphatic methyl resonances at 1.45(s, 3H), 1.57(s, 3H), and 1.79ppm(s,3H), and an acetyl methyl resonance at 2.20 ppm suggested a non-symmetrical alicyclic ring similar to that proposed for the diacetate of 2-hydroxy garvin A (120), a metabolite whose structure had previously been elucidated (see page 91) using a combination of nOe and X-ray data. Methyl
resonances at 1.50, 1.60 and 1.70 ppm in the $^1$H NMR spectrum of 120 correspond closely to those in 98, leading to the proposed structure for 22. An attempt to confirm this assignment by analysis of the $^{13}$C spectrum (75 MHz, CDCl$_3$) of the triacetate 98 led to considerable confusion, since only two of the three ketone carbons and six of the eight methyl carbons could be observed. Changing solvents (DMSO-d$_6$, C$_6$D$_6$, acetone-d$_6$), temperature and delay times did not alleviate this problem. However, treatment of 98 with pyridine at room temperature converted it to the diacetate (99), whose $^1$H and $^{13}$C NMR spectra were entirely consistent with the proposed structure. Reacetylation of the diacetate with acetic anhydride/pyridine regenerated the triacetate 98.

2-Hydroxygarveatin A (22) appears to be formed by C2 hydroxylation of garveatin A (77). It is interesting to speculate whether this reaction occurs by aerial oxidation during the storage period and purification steps, or whether the process is enzymatically controlled in-vivo. The presence of relatively large amounts of 2-hydroxygarveatin A in fresh extracts of G. annulata, prior to any chromatography, seems to indicate that the compound is not an artifact produced by oxidation of garveatin A, and that it occurs as a metabolite in the intact organism.

In order to carry out an in-vitro conversion of garveatin A to 2-hydroxygarveatin A, it was decided to make use of lead tetraacetate$^{85}$ to introduce an oxygen atom in the form of an acetoxy group at the C2 position of garveatin A. First, the C8 and C9 OH groups were protected as their methyl ethers (Scheme 2). This was achieved by treatment of 77 with dimethyl sulfate to
Scheme 2.
give trimethylgarveatin A (80) as previously described. The 3-methoxy group was then hydrolised preferentially, by refluxing in 50% aqueous acetic acid to yield the 8,9-dimethyl derivative 100. When this compound was stirred with Pb(OAc)₄ in glacial acetic acid at room temperature overnight, the major reaction product was the 2-acetoxy derivative 101. The $^1$H NMR spectrum (80 MHz, CDCl₃) of 101 contained methyl singlets at 1.47, 1.57 and 1.76 ppm as well as an acetate resonance at 2.23 ppm, which had almost identical chemical shifts to the corresponding resonances in 2-hydroxygarveatin A triacetate (88). The UV maxima of both compounds were very similar, indicating the presence of the same type of chromophore.

(vi) 2-Hydroxygarveatin B.

The less polar metabolite present in the flash fraction eluted with 100% ethyl acetate was purified by LH-20 chromatography (90% MeOH/CH₂Cl₂) to give 40 mg of a yellow solid, whose molecular formula was determined to be C₂₀H₂₂O₅ by HRMS (m/z observed 342.1467, req'd 342.1468). The chemical shifts of the three methyl groups in the $^1$H NMR (80 MHz, CDCl₃) spectrum (1.52, 1.65, 1.81ppm) corresponded closely to those in the alicyclic ring of 2-hydroxygarvin A (112) (1.52, 1.66, 1.81ppm)(see page 95). Furthermore, the remaining proton resonances bore a close resemblance to those for the aromatic nucleus and its substituents in garveatin B (88). This $^1$H NMR data, in conjunction with the molecular formula, suggested that this compound was 2-hydroxygarveatin B (102). Evidently,
102 \( R, R_1 = H \)
103 \( R = H, R_1 = Ac \)
104 \( R, R_1 = Ac \)
105 \( R = CH_3 CO^- \)
106 \( R = CH_3 CH_2^- \)
107
of an acetyl group with an ethyl side-chain causes a substantial increase in hydrophobicity, resulting in a higher $R_f$ value and greater solubility in chloroform of 102 compared to 97. A similar difference in polarity was noticed between garveatin A (77) and garveatin B (88).

Treatment of 102 with acetic anhydride and pyridine yielded a mixture of the diacetate (103) and the triacetate (104) as the major reaction products. In this case the diacetate contained acetoxy groups at the C8 and C9 positions, as evidenced by the chemical shifts of the aromatic protons at 7.62 and 7.66 ppm in the $^1$H NMR spectrum (80 MHz, CDCl$_3$), as well as a broad singlet at 4.63 ppm, due to the C2 OH group. This is in contrast to the substitution pattern of the diacetate of 2-hydroxy garveatin A (97) which contains acetoxy groups at the C2 and C8 positions.

(vii) Quinone derivatives of the Garveatins

A second class of oxidized derivatives of the garveatins is represented by the substituted naphthoquinones, garveatin A quinone (105) and garveatin B quinone (106). These compounds are present in low concentration (<5%) relative to the parent garveatins, but because of their bright red colour, they are easily detected in the crude extracts.

The ethyl acetate phase of the G. annulata extract was subjected to suction-flash chromatography using an ethyl acetate/CH$_2$Cl$_2$ gradient to give a deep red fraction which was further purified by preparative TLC (2% MeOH/CH$_2$Cl$_2$) to give 11mg of garveatin A quinone (105) as a red oil. This compound was also
present in the hexane and CH₂Cl₂ phases, but was more difficult to purify from these extracts due to the presence of other components with approximately the same Rf values.

Quinone 105 was shown by HRMS to have a molecular formula of C₂₀H₁₈O₆ (m/z obs'd 354.1108, req'd 354.1103) and its UV spectrum (in MeOH) and bright red colour was typical of anthraquinone-type compounds. The ¹H NMR spectrum of 105 suggested a close relationship to garveatin A (77) and resonances at 1.98 (s, 3H), and 1.63 ppm (s, 6H) could be assigned to a methyl group at C2 and a gem-dimethyl at C4 respectively. In addition, singlets at 2.60 (3H) and 2.41 ppm (brs, 3H) were appropriate for an aromatic acetyl at C7 and an aromatic methyl substituent at C6. However, only one aromatic proton was present in the ¹H NMR spectrum of 32 and its chemical shift (7.55 ppm) corresponded closely to that of the C4 proton (7.57 ppm) in the anthraquinone (107) prepared by Marini-Bettolo's group.86

The ¹³C NMR spectrum (75 MHz, CDCl₃) of 105 contained resonances at 193.0 and 180.4 ppm which were appropriate for the quinone carbonyls of the proposed structure.

The presence of two phenolic proton resonances at 11.45 and 11.66 ppm indicated that 105 exists as the 3-keto tautomer in CDCl₃. In this form, the 1-OH group can form a H-bond with the C9 quinone carbonyl oxygen atom, and this chelating effect is responsible for the downfield shift of the 1-OH proton.

Garveatin A quinone (105) was present in fresh extracts (< 48 hours) of G. annulata, indicating that it may well be a true metabolite, and not just an oxidation artifact of garveatin A. Treatment of garveatin A under a stream of air for 16 hours in a
1:1 mixture of 1N NaOH and MeOH gave a surprisingly low yield of the quinone 105 (10%), proving that even under vigorous oxidation conditions, this conversion is not very facile. Similarly, when 77 was allowed to stand overnight in a methanolic slurry of silica-gel in the presence of either HCl or NaOH, very low yields (<5%) of the quinone were obtained.

Garveatin B quinone (106) has also been detected in extremely small (<1 mg) amounts in samples of garveatin B which have been allowed to stand in solution. This compound has not been observed in the crude G. annulata extracts so there is no firm evidence that it is a bona fide natural product. However, oxidation of garveatin B to garveatin B quinone occurs in CDCl₃ solution at room temperature. Treatment of pure garveatin B (2 mg) with CDCl₃ (1 ml), and analysis of this sample on normal phase HPLC (50% EtOAc/Hexane) at various time-intervals showed the formation of garveatin B quinone, the concentration reaching a maximum (68% of total) after 50 minutes. Quinone 106 was obtained from this reaction as a red oil with an Rf of 0.63 in the standard TLC system and it had a molecular formula of C₂₀H₂₀O₅ (m/z 340.1306, req'd 340.1311). Its ¹H NMR (400MHz, CDCl₃) spectrum was almost identical to that of garveatin A quinone (105), except for the presence of resonances at 1.16 (t,J=7Hz, 3H) and 2.78 ppm (q,J=7Hz,2H) due to an aromatic ethyl group, and the absence of an aromatic acetyl resonance around 2.6 ppm. This compound also appears to exist as the 3-keto tautomer (in chloroform solution) since both the OH protons (11.79 and 11.84 ppm) are quite far downfield.
The first evidence of the presence of dimers in the *G. annulata* extracts came when analysis of the $^1$H NMR spectrum (400 MHz, CDCl$_3$) of garveatin B indicated the presence of a second set of resonances whose intensity increased with time to give a 1:2 ratio to 88 after 24 hours. Subsequent repurification of the NMR sample by preparative TLC (40% ethyl acetate/hexane) yielded a second less polar compound ($R_f$ 0.67 in standard TLC system) whose NMR spectrum (CDCl$_3$) contained signals corresponding to the "extra" resonances in that of 88. Thus, 88 had partially decomposed to another compound in CDCl$_3$ solution. On standing in acetone-$d_6$ or C$_6$D$_6$ solutions, no such decomposition was detected, either by NMR or TLC. The decomposition product, which had previously been identified as a very minor constituent of the flash fraction containing garveatin B, had a UV spectrum which showed strong similarities to that of 2-hydroxy garveatin B (102) in both neutral and basic solution.

The $^1$H NMR spectrum (400 MHz, CDCl$_3$) contained resonances at 1.18 (t, $J$=7Hz, 3H), 2.80 (q, $J$=7Hz, 2H), 2.43 (brs, 3H), 6.98 (s,2H), 9.38 (s,1H) and 15.83 (s,1H) ppm, appropriate for aromatic ethyl, aromatic methyl, two aromatic and two phenolic protons respectively. This suggested that the aromatic portion of garveatin B was present in the molecule, but it was interesting to note that all the above resonances were shielded in comparison to those in garveatin B, by amounts varying from 0.03 ppm for the C14 methyl to 0.40 ppm for the C9 OH proton. The remaining resonances in the $^1$H NMR spectrum were methyl singlets at 1.38,
1.66 and 1.88 ppm, indicating that the alicyclic ring now contained an asymmetric centre. Due to the limited amount of sample, it was not possible to obtain good $^{13}$C spectral data, but a very weak spectrum (75 MHz, $C_6D_6$) did indicate resonances at 202.4 and 209.0 ppm, appropriate for ketone carbons at C1 and C3 respectively. These correspond closely to the C1 and C3 carbons in garveatin C (93) (205.8 and 211.6 ppm). A resonance at 63.0 ppm in the spectrum of the decomposition product has almost the same chemical shift as the C2 carbon (64.1 ppm) in 93. This suggests that the C2 position in this compound is also dialkylated.

The EI mass spectrum of the decomposition product gave a highest mass ion at 326 amu, suggesting that it was a structural isomer of 88. However, the major product obtained by acetylation with acetic anhydride/pyridine, which contained acetoxy groups at the C8 and C9 positions as evidenced by the chemical shifts of the two aromatic protons (7.61, brs,1H and 7.75, s,1H), gave a weak molecular ion at 818 amu in the mass spectrum. It was concluded that the decomposition product had structure 108, produced by dimeric association of two garveatin B monomers. The mass spectrum of the acetate (109) also showed weak fragment ions at 776 and 734 amu, corresponding to successive losses of two acetate units from the parent ion. A much more intense peak at 410 daltons corresponds to cleavage of the 2,2' carbon-carbon bond and transfer of a hydrogen to form an ion corresponding to the diacetate of garveatin B. Further losses of 42 daltons from this daughter ion gave intense peaks at 368 and 326 daltons. By
108  R = H  

109  R = Ac

110  R = H  

111  R = Ac

112

113
analogy, the peak at 326 daltons in the mass spectrum of the underivatized decomposition product 108 was in fact the daughter ion formed by an extremely facile cleavage of the central bond and transfer of a hydrogen atom. No peak at 650 daltons could be detected in the EI mass spectrum of 108. Evidently, the acetylated derivative of the dimer is more stable (or more volatile) in the mass spectrum and it can therefore can detected at a high gain setting. There is ample precedent for the above observations, since it is well established that principal ions in the mass spectra of bianthrones are daughter ions of mass M/2+1 for symmetrical dimers65.

The corresponding dimer of garveatin A was not observed when this compound was allowed to stand in CDCl₃ solution. In an attempt to prepare this dimer, more vigorous conditions were employed. Treatment of 77 with conc. HCl in CH₃CN solution under a stream of air gave in low yield (10%) a product which was purified by preparative TLC in 5% MeOH/CHCl₃. The highest mass ion in the EI mass spectrum occured at 340 daltons, appropriate for cleavage at the 2,2'bond to generate the monomer, as previously discussed for 108. The ¹H NMR spectrum (300 MHz, CDCl₃) contained resonances assignable to the naphthalene portion of garveatin A, and as in the case of garveatin B dimer, their chemical shifts were upfield compared to the values in the spectrum of 77. Three-proton singlets at 1.47, 1.59 and 1.94 ppm, due to methyl groups at the C2 and C4 (2) positions, completed the ¹H NMR spectrum.

More concrete proof of the dimeric nature of garveatin A
dimer (110) was obtained by acetylation with acetic anhydride and pyridine to yield the tetraacetate 111. A weak peak at 846 daltons was observed in the mass spectrum of 111, in addition to peaks at 804, 762, 720 and 678 daltons due to successive losses of the four acetate groups. As was the case for the tetraacetate of garveatin B dimer (109), resonances at 7.64 (brs, 1H) and 7.79 ppm (s,1H) in the $^1H$ NMR spectrum of 111 reflected acetylation at the C8 (C8') and C9 (C9') positions of 110.

In principle, dimerisation at the C2 position of garveatin A could lead to the meso form 112 and also to a racemic (+,-) mixture of the R,R and S,S forms 113. Since the meso form is diastereomeric with either enantiomer of the racemate, it would be expected to have different chemical and spectral properties and also a different $R_f$ value from that of the racemic form.

Cameron and co-workers 87 have separated the meso and (+,-) diastereomers of 10,10' emodin bianthrone (114) and identified each by using a chiral NMR shift reagent. They found that on addition of tris[3-heptafluoropropylhydroxymethylene- (+) camphorato] europium III to a solution of the hexaacetate (115) of one diastereomeric form, the 10,10' proton singlet in the $^1H$ NMR spectrum was split into two lines of equal intensity. Since the two enantiomers could display different chemical shifts when in contact with the chiral reagent, this diastereomer was formulated as the racemic mixture. Addition of the shift reagent to the hexaacetate of the other diastereomer did not induce any splitting in the $^1H$ NMR lines, implying that this was the meso form of the bianthrone.

It was anticipated that treatment of the tetraacetate (111)
114 \( R = H \)

115 \( R = Ac \)

116

117

118
of garveatin A dimer with the same chiral shift reagent would enable us to (a): determine whether one or both stereoisomeric forms were present in the sample, and (b): distinguish between the meso and (+,-) forms if only one type of stereoisomer was present. However, on titration of 111 with the shift reagent, no changes in the chemical shifts or multiplicity of the proton signals was observed. Thus, it appeared that the reagent was not complexing with the dimer and consequently no conclusions could be drawn from the experiment.

(ix) Desacetyl derivatives

Treatment of garveatin A (77) with strong acid provided an indication of the lability of the aromatic acetyl group under these conditions. When 77 was refluxed for 10 minutes in the presence a 1:1 (v/v) mixture of conc. HCl and glacial acetic acid, a less polar product was obtained in approx. 50% yield. This compound had a molecular weight of 298 daltons corresponding to a loss of 42 daltons from 77. Its 1H NMR spectrum (300 MHz, CDCl3) contained three aromatic protons, and a methyl singlet in the region of 2.60 ppm was absent. The rest of the spectrum was almost identical to that of 77, indicating that the aromatic acetyl group had been lost to give structure 116. Two of the aromatic protons at 7.00 and 6.68 ppm were broad singlets which collapsed to doublets with 1.6 Hz coupling on irradiation of the aromatic methyl group at 2.43 ppm. Therefore, these resonances were assigned to the protons at C5 and C7 respectively, the C7 resonance being more shielded due to its beta position on the
naphthalene nucleus. Also present in the $^1$H NMR spectrum of 116 were resonances corresponding to the diketo tautomer in a 2:5 ratio with those of the keto-enol form. It is interesting to note that no keto-enol tautomerism was evident in the CDCl$_3$ spectrum of 77, this molecule being present only as the 1-keto, 2,3-enol form.

When garveatin A quinone (105) was treated with CH$_3$COOH/HCl, desacetyl garveatin A quinone (117) was formed, as evidenced by a molecular ion at 312 daltons in the mass spectrum, and the appearance of another aromatic proton (7.11 ppm, brs, 1H) in the $^1$H NMR spectrum (300 MHz, CDCl$_3$) in place of an aromatic acetyl resonance. As in the previous case, irradiation of the aromatic methyl group at 2.48 ppm collapsed the broad C5 and C7 protons to sharp doublets (J=1.6 Hz), and established the meta orientation of these hydrogens. In contrast to desacetyl garveatin A (116), compound 117 did not exhibit any of the diketo form in the $^1$H NMR spectrum, suggesting a very strong H-bonding interaction between the Cl OH group and the quinone carbonyl.

The hydrolysis of the aromatic acetyl group is analogous to the hydrolysis of the n-butyryl group of rhodocomatulin dimethyl ether (40), a pigment isolated from a species of marine crinoid by Sutherland's group$^{54}$. In this case, butyric acid was also isolated and identified by gas and paper chromatography. Sutherland postulated that the elimination occurs via a reverse Friedel Crafts acylation reaction, and by analogy, a likely intermediate in the case of the garveatin A reaction would be structure 118, resulting from attack of a proton at C7.
(x) **Biosynthesis**

The garveatins are typical examples of polyketide-derived metabolites. It appears likely that all the garveatins are derived from the same nonaketide precursor which has been dimethylated at C8 and either mono- or dimethylated at C10 (Scheme 3). The folding pattern shown can lead to garveatins A and D via multiple aldol condensations and dehydrations. O-Methylation of garveatin D can give garveatin C, whereas reduction of the aromatic acetyl group of garveatin A produces garveatin B, although it is conceivable the latter step could occur before cyclisation. Another possible route to garveatin B may involve a precursor containing a butyric acid starter unit. Also, in view of the fact that the C2 position of garveatin A has been shown to be highly susceptible to alkylation, it is possible that methylation of garveatin A (i.e. after cyclisation) could be the final step in garveatin D biosynthesis.
Scheme 3.
Figure 6. 80 MHz $^1$H NMR spectrum of 80 in CDCl$_3$. 

Chemical structure of compound 80 with labels.
Figure 7. 80 MHz $^1$H NMR spectrum of 88 in CDCl$_3$. 
Figure 8. 80 MHz $^1$H NMR spectrum of 93 in CDCl$_3$. 
Figure 9. 270 MHz $^1$H NMR spectrum of 96 in CDCl$_3$. 
Figure 10. 80 MHz $^1$H NMR spectrum of 98 in CDCl$_3$. 
Figure 13. 270 MHz $^1$H NMR spectrum of 108 in CDCl$_3$. 
Figure 14. 100 MHz $^{13}$C NMR spectrum of 93 in CDCl$_3$. 
Figure 15. 75 MHz $^{13}$C NMR spectrum of 105 in CDCl$_3$. 
B. THE GARVINS

(i) 2-hydroxygarvin A

A second family of anthracenones whose carbon skeleton differed from that of the garveatins was discovered on further examination of the methanol extracts of *G. annulata*. This new family, named the garvins, was first recognised through a detailed study of 2-hydroxygarvin A (119). This metabolite was obtained in reasonably large amounts (100mg/collection), was relatively easy to purify and was highly soluble in CDCl₃, making it well suited to NMR studies.

TLC analysis of the crude hexane and methylene chloride phases of the *G. annulata* extracts indicated the presence of a component which had a white fluorescence under long-wave UV. This compound eluted with 50% ethyl acetate/hexane from the silica-gel vacuum flash column and was further purified on LH-20 (90% MeOH/CH₂Cl₂). A final fractionation on preparative TLC (10% ethyl acetate/CHCl₃) gave pure 2-hydroxygarvin A (119), which crystallised from diethyl ether as yellow needles (m.p. 195-197°C).

Compound 119 had a molecular formula of C₂₃H₂₆O₇ (M⁺ 414.1677, calc'd 414.1679) and a UV spectrum with maxima at 226, 278, 318, 332, and 385 nm, indicative of an anthracenone chromophore. The ¹H NMR spectrum (400 MHz,CDCl₃) contained resonances at 6.98 and 7.21 ppm, which corresponded closely to the chemical shifts of the H5 and H10 protons in garveatins A-D.
A singlet (1H) at 14.25 ppm was appropriate for a phenol proton hydrogen-bonded to a carbonyl oxygen.

Acetylation of (119) with acetic anhydride and pyridine formed the diacetate 119 which contained new 1H NMR resonances at 2.25(s,3H) and 2.48 ppm(s,3H) that could be assigned to one aliphatic and one phenolic acetate respectively. The downfield shift of the aromatic proton at 7.21 in 119 to 7.73 ppm on acetylation required that this proton be ortho or para to the phenol. Also, the absence of carbinol methine or methylene protons in the 1H NMR spectrum of 119 indicated that the aliphatic alcohol was tertiary. A 13C NMR resonance at 81.6 ppm (s), reflecting the deshielding influence of an alcohol oxygen atom, supported this assignment.

The presence of an n-propyl group was inferred from the 1H NMR spectrum of 119. Resonances at 1.04 (3H,t,7Hz), 1.73 (m,2H) and 3.15 (m,2H) were shown by decoupling experiments to comprise an isolated seven spin system appropriate for a three carbon chain attached to an aromatic nucleus. Irradiation of the two-proton multiplet at 1.73 ppm collapsed the triplet at 1.04 ppm to a singlet, and the multiplet at 3.15 ppm to an AB system with 12 Hz coupling. This showed that the two benzylic protons were not chemical shift equivalent, differing by 0.05 ppm. Irradiation of the three-proton triplet at 1.10 ppm collapsed the multiplet at 1.73 ppm to a doublet of doublets (J=7,8Hz) whereas irradiation of the multiplet at 3.15 collapsed the same resonance to a quartet (J=7Hz). Resonances at 36.0 (t), 25.3 (t) and 14.7 ppm(q) in the gated decoupled 13C NMR spectrum were assigned to the three carbons of the n-propyl side-chain. Methyl resonances at
3.98 and 3.99 ppm (both 3H, s) were assigned to an aromatic methyl ether ($^{13}$C NMR: 55.9, q) and a methyl ester ($^{13}$C NMR: 52.3, q; 168.3, s). Further evidence for the methyl ester came from a prominent mass spectral fragment at m/z 381 (M$^+$-31) due to loss of an $\text{OCH}_3$ group. The remaining $^1$H NMR resonances at 1.52, 1.66 and 1.81 (all 3H, s) could be attributed to three aliphatic methyl groups. A gated decoupled $^{13}$C NMR spectrum (100MHz, CDC$_3$) of 119 contained two quartets of quartets (J = 160, 4 Hz) at 28.2 and 30.6 ppm, indicating that two of the aliphatic methyls were attached to the same carbon. The chemical shifts of these methyl groups were close to those of the C12 and C13 carbons (28.5 ppm) in the $^{13}$C spectrum of garveatin C (93), which suggested that 119 might also contain a gem-dimethyl substituent at C4. This assignment was supported by the presence of a singlet at 46.9 ppm comparable to the C4 resonance at 48.1 ppm in the spectrum of garveatin C. A resonance at 209.9 ppm (s) was assigned to an unconjugated ketone, which accounted for the remaining oxygen atom in the molecular formula.

The above spectral data was consistent with a 1-anthracenone skeleton containing phenol, tertiary alcohol, n-propyl, methyl ester, methyl ether, ketone, methyl (3x) and aromatic proton (2x) functionalities. A combination of NMR experiments and biogenetic arguments was employed to determine the placement of substituents on the anthracenone nucleus. A difference nOe experiment in which the aromatic singlet at 7.21 ppm in the $^1$H NMR spectrum of 119 was irradiated, produced nOe enhancements in the other aromatic proton at 6.98 ppm and also in the methyl resonances at 1.52 and
119  \( R = H \)  120  \( R = Ac \)

121

122  \( R = H \)  123  \( R = Ac \)

124  \( R = H \)  125  \( R = CH_3 \)
1.81 ppm. This result is consistent with placement of the gem-dimethyl group at C4, the phenolic proton at C9 and aromatic protons at C10 (7.21ppm) and C5 (6.98ppm). Irradiation of the methyl singlet at 1.52 ppm generated nOe's in the methyl singlet at 1.81 and the aromatic proton at 7.21 ppm. Similarly, irradiation at 1.81 ppm produced an enhancement of the resonances at 1.52 and 7.21 ppm. Neither of the last two irradiations produced an observable enhancement in the methyl singlet at 1.66 ppm. On the basis of these experiments a ketone was placed at C3, and aliphatic methyl and tertiary alcohol substituents at C2. The aliphatic methyl resonance at 29.0 ppm in the gated $^{13}$C NMR spectrum did not show any long-range coupling which was consistent with attachment at the C2 carbon. A close correspondence between the C3 ketone resonance at 209.9 ppm and the C3 resonance at 211.6 ppm in the spectrum of garveatin C provided further evidence for a six-membered alicyclic ring in 119. The remaining n-propyl, methyl ether and methyl ester substituents had to be attached to carbons C6-C8. There are only two arrangements of these groups, represented by structures 119 and 121, which are consistent with straight-forward polyketide biogenesis. Irradiation of the aromatic proton at 6.98 ppm (H5) induced an nOe in the methyl resonance at 3.98 ppm, which led us to propose 119 as the correct structure. Furthermore, acetylation of the C9 OH group shifted the C15 methylene protons of the n-propyl side-chain upfield by 0.31 ppm (3.15,m, in 119; 2.94,m, in 121). This result was consistent with placement of the n-propyl group at C8, where it is in close spatial proximity to the C9 OH
Assignment of the rest of the $^{13}$C NMR spectrum of 119 was aided by analysis of the gated decoupled spectra run in CDCl$_3$ and in CDCl$_3$ with added D$_2$O. A doublet of doublets at 105.0 ppm, showing a large one-bond coupling of 160 Hz and a small three-bond coupling of 6 Hz (to the H10 proton) was assigned to the C5 position, since this resonance is shielded by the ortho methoxy group at C6. The other doublet of doublets at 116.5 ppm (160,6 Hz) was assigned to the C10 carbon which showed three-bond coupling to the C5 proton. The planar geometry of the C-H and C-C bonds in aromatic molecules gives rise to three-bond C-C-C-H couplings in the 6-8 Hz range, which are usually much larger than two-bond C-C-H couplings.

The resonance at 107.3 ppm, assigned to the C9a position, appeared as a doublet of doublets ($J=8, 4.8$ Hz) in the gated spectrum run in CDCl$_3$, but collapsed to a doublet on addition of D$_2$O. This indicated that the C9 OH proton is coupled to C9a ($J=4.8$ Hz), and this coupling is removed on exchange of that proton with D$_2$O. This leaves a three-bond 8 Hz coupling to the H10 proton. The resonance at 116.7 ppm, assigned to the C8a position, appeared as a broad, distorted doublet in the gated spectrum in CDCl$_3$, but collapsed to a broad singlet on addition of D$_2$O. As in the previous case, this implies a three-bond coupling to the C9 OH proton. The broadness of the resonance in the D$_2$O exchanged spectrum probably reflects three-bond coupling to both H5 and H10 protons, as well as to one or both H15 protons on the n-propyl side-chain. Finally, the resonances at 199.9 and 209.9 ppm, assigned to the C1 and C3 carbonyl groups respectively collapsed.
from broad doublets \((J=2.9, \ 2.0\text{Hz resp.})\) to broad singlets on addition of \(\text{D}_2\text{O}\). This indicates a long-range coupling to the \(\text{C}_2\) \(\text{OH}\) group, and the smaller \(J\) value may be a consequence of the non-planarity of the \(\text{H}-\text{O}-\text{C}-\text{C}\) bond sequence.

At this stage there was still some ambiguity over the assignment of \(\text{C}_5\) and \(\text{C}_{10}\) \((105.0\ \text{or}\ 116.5\ \text{ppm})\) and also \(\text{C}_{8a}\) and \(\text{C}_{9a}\) \((107.3\ \text{or}\ 116.7\ \text{ppm})\). The use of a novel experiment\(^{89,90}\) called INAPT (Insensitive Nuclei Assigned by Polarisation Transfer) provided the crucial assignment information. This technique, developed recently by Bax, provides long-range heteronuclear connectivity information by using a set of low intensity radiofrequency pulses to irradiate a preselected proton resonance. This pulse sequence can then transfer proton magnetisation to any \(^{13}\text{C}\) nucleus that has a significant long-range scalar interaction with this proton. The major advantage of this technique over other methods is that this long-range connectivity is obtained with high sensitivity, on the order of the normal \(^1\text{H}\) decoupled spectrum. Therefore the INAPT experiment can be performed on much more dilute samples than is currently possible using the 2-D HETCOR\(^91\) technique. INAPT also requires a much smaller data base since it is a "1-D" experiment. Another advantage of the INAPT technique is its selectivity, only one proton resonance is irradiated at a time and consequently only one set of correlations are observed. Figure 16 shows the decoupled spectra obtained with INAPT transfers from \(\text{H}_5\) and \(\text{H}_{10}\). Since \(3J_{\text{CH}}\) couplings in aromatic molecules are in the order of 6-8 Hz, the experiment was optimised for transfer through 7Hz
Figure 16. 75 MHz INAPT spectra of 119 in CDCl₃
couplings. Irradiation of the H5 proton (6.98 ppm) resulted in transfer of magnetisation to resonances at 116.5, 116.7 and 126.9 ppm. Since the resonance at 126.9 ppm had previously been assigned to C7, it follows that the C10 and C8a resonances are at 116.5 and 116.7 ppm respectively. Similarly, irradiation of H10 (7.21 ppm) produced a transfer to resonances at 46.9 (C4), 107.3, 116.7 (C8a) and 105.0 ppm. Therefore, C5 (105.0) and C9a(107.3) were positively assigned. The low-intensity peak at 142.1 ppm in this spectrum is probably due to the presence of a significant four-bond long-range coupling between H10 and C8 (see ref. 90). A similar argument can be used to explain the presence of a weak resonance at 107.3 ppm (C9a) when H5 is irradiated. Both spectra were acquired with 800 scans, requiring approximately 30 minutes each, with a 60 mg sample size.

Support for the proposed structure of 2-hydroxygarvin A came from its similarity to garvin A quinone (122), whose structure was solved by an X-ray diffraction analysis on its monoacetate (123).

(ii) Garvin A quinone

Close examination of the fractions eluting with 50% ethyl acetate/hexane and 100% ethyl acetate by flash chromatography enabled us to identify a minor compound, whose bright red color was reminiscent of garveatin A quinone (105). Sequential purification by LH-20 (90% MeOH/CH₂Cl₂) chromatography, preparative TLC (50%ethyl acetate/hexane) and normal-phase HPLC (ethyl acetate/hexane) yielded 10 mg of an orange-red solid. Mass spectrometry indicated that it had a molecular formula of
\[ \text{C}_{23}\text{H}_{24}\text{O}_7 \ (M^+ \ 412.1518, \ \text{calc'd 412.1522}) \]. \text{H} NMR analysis (see Table 7) showed that it contained the n-propyl, methyl ether and methyl ester substituents present in 2-hydroxygarvin A (119). Singlets at 1.61 (6H) and 1.98 ppm (3H) were almost identical to the resonances assigned to the C11-13 methyl groups in the \text{H} NMR spectrum of garveatin A (77), suggesting the presence of the same alicyclic ring in both compounds. Acetylation with acetic anhydride and pyridine produced a monoacetate which crystallised from acetone, and whose structure was shown to be 123 by single-crystal X-ray diffraction analysis, performed by Van Duyne and Clardy at Cornell. Therefore the parent compound, named garvin A quinone, had structure 122. A computer-generated perspective drawing of 123 is given in Figure 17, and exhibits a pronounced non-planarity of the quinone ring which adopts a boat-like conformation with the C9 and C10 carbons above the plane of the ring. An enolic resonance at 12.15 ppm (s,1H) in the \text{H} NMR spectrum of 122 indicates hydrogen-bonding between the C1 OH proton and the C9 carbonyl oxygen. Thus, 122 exists as the 3-keto tautomer in chloroform solution, as is the case for garveatin A quinone (105) and garveatin B quinone (106).

(iii) Garvin A

By analogy with the garveatin family, it was anticipated that the \textit{G. annulata} extracts might contain a garvin-type compound having a keto-enol functionality in the alicyclic portion of the molecule, similar to that in garveatins A and B. This prediction was verified by purification of garvin A (124).
Figure 17. Computer generated X-ray structure of the acetate of garvin A quinone (123).
using LH-20 chromatography (90% MeOH/CH₂Cl₂) and preparative TLC (10% ethyl acetate/CHCl₃) from the flash fractions eluted with 100% ethyl acetate. Compound 124 was obtained as a bright yellow solid that gave a parent ion in the mass spectrum at m/z 398, appropriate for a molecular formula of C₂₃H₂₆O₆. Its ¹H NMR spectrum showed resonances for the n-propyl (1.03, t, J=7Hz, 3H; 1.73, m, 2H; 3.18, m, 2H), methyl ether, methyl ester (3.95, s, 3H; 3.79, s, 3H), H5 (6.91, s, 1H) and H10 (7.17, s, 1H) functionalities found in 2-hydroxygarvin A (119). Additional resonances at 1.64 (s, 6H), 1.99 (s, 1.5H), 1.50 (d, J=7Hz, 1.5H) and 4.01 (q, J=7Hz, 0.5Hz) corresponded closely to the signals assigned to the tautomeric forms of ring A in garveatin B (88). A simple combination of the two portions indicated by ¹H NMR allowed the assignment of structure 124 to garvin A. The tautomeric forms were present in approximately 1:1 ratio as measured by peak intensities in the ¹H NMR spectrum.

Treatment of garvin A with diazomethane yielded 3-methyl garvin A (125) as the major reaction product. A ¹H NMR resonance at 3.93 ppm (s, 3H) was appropriate for a methyl ether, and the presence of the hydrogen-bonded C9 OH singlet at 15.87 ppm indicated that the C3 OH group had been methylated. This result is analogous to the methylation of garveatin B (88) at the C3 OH position with diazomethane.

(iv)Garvin B

The second most abundant polyketide-type metabolite present in the G. annulata extracts was found to be garvin B (126). This
Garvin B (126) was shown by HRMS to have a molecular formula of C_{21}H_{20}O_{6} (obsd 368.1269, reqd 368.1260) and it gave a bright yellow fluorescence under long-wave UV when TLC'd on silica-gel. Methyl resonances at 1.54, 1.58 and 1.85 ppm (all s, 3H) in the $^1$H NMR spectrum (300 MHz, DMSO-d$_6$) of 126 showed a close resemblance to the resonances for the methyl groups in the alicyclic ring of garveatin A (77) (DMSO-d$_6$: 1.59, s, 6H; 1.90, s, 3H), indicating that the two molecules had this substructure in common. Resonances at 7.16 and 7.35 ppm could be assigned to aromatic protons at C5 and C10 of an anthracenone skeleton by analogy with other Garveia metabolites. The presence of two phenolic groups in the molecule was indicated by singlets at 11.14 and 17.85 ppm in the $^1$H NMR spectrum and verified by acetylation with acetic anhydride/pyridine to yield the 3,6-diacetate (127) and the 3,6,9-triacetate (128) as the major reaction products. A six proton spin system (1.50, d, J=7Hz, 3H; 4.77, m, 1H; 3.23, dd, J=19, 13Hz, 1H; 4.45, dd, J=19, 3Hz, 1H) was assigned to an n-propyl residue which contained an oxygen substituent on the central carbon and an aryl substituent at one of the terminal carbons. Decoupling experiments were performed on the trimethyl derivative (129), formed by treatment of garvin B.
126  $R, R_1 = H$
127  $R = Ac, R_1 = H$
128  $R, R_1 = Ac$

129
with methyl iodide, since this compound was soluble in CDCl$_3$ and showed better resolution in the $^1$H NMR spectrum. In addition, a spectrum of the above-mentioned spin-system in 129 was generated using a spin-simulation program (Figure 21a), and the simulated spectrum closely matched the appropriate resonances in the actual $^1$H NMR spectrum.

The $^{13}$C NMR spectrum (75 MHz, DMSO d$_6$) of garvin B contained resonances at 20.4, 75.4, 34.0 ppm which were assigned to the substituted propyl residue, the most deshielded resonance being appropriate for an oxygen-bearing methine carbon. A resonance at 170.6 ppm indicated an ester functionality in the molecule. Modification of the structure of garvin A (124) by replacing the methoxy substituent at C6 with a phenol, and formation of a delta lactone between an alcohol at C16 and a carboxylic acid group at C14 leads to structure 126 which effectively accounts for all the properties of garvin B. This molecule contains a chiral center at C16 and is optically active, as evidenced by the specific rotation measurement of $[\alpha]_D^{25} +172.69^\circ$ (c 0.26, CHCl$_3$) for the trimethyl derivative 129. This chirality accounts for the non-equivalence of the gem-dimethyl groups in the NMR spectra of garvin B. Compound 129 has been methylated at the C2 position as well as at the C6 and C9 OH groups, analogous to formation of 2,8,9-trimethyl garveatin A (25) from garveatin A with methyl iodide.
Table 5: $^1$H NMR data (CDCl$_3$) for the Garvins.

Chemical shifts in ppm from TMS.

<table>
<thead>
<tr>
<th>H on C#</th>
<th>Garvin A $^{124}$</th>
<th>2-OH Garvin A $^{119}$</th>
<th>Garvin B$^a$ $^{126}$</th>
<th>2-OH Garvin B $^{130}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>20H</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>4.02 br s</td>
</tr>
<tr>
<td>5</td>
<td>6.91</td>
<td>6.98</td>
<td>7.16</td>
<td>7.11(7.11)</td>
</tr>
<tr>
<td>60R</td>
<td>3.95*</td>
<td>3.98*</td>
<td>11.14*</td>
<td>11.44(11.45)</td>
</tr>
<tr>
<td>10</td>
<td>7.17</td>
<td>7.21</td>
<td>7.35</td>
<td>7.11(7.11)</td>
</tr>
<tr>
<td>11</td>
<td>1.99</td>
<td>1.66</td>
<td>1.85</td>
<td>1.65(1.66)</td>
</tr>
<tr>
<td>12</td>
<td>1.64</td>
<td>1.52</td>
<td>1.54</td>
<td>1.50(1.54)</td>
</tr>
<tr>
<td>13</td>
<td>1.64</td>
<td>1.81</td>
<td>1.58</td>
<td>1.80(1.82)</td>
</tr>
<tr>
<td>140R</td>
<td>3.97*</td>
<td>3.99*</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>15</td>
<td>3.18 m</td>
<td>3.15 m</td>
<td>3.23 dd</td>
<td>3.41(3.44)dd</td>
</tr>
<tr>
<td>16</td>
<td>1.73 m</td>
<td>1.73 m</td>
<td>4.77 m</td>
<td>4.77(4.77)m</td>
</tr>
<tr>
<td>17</td>
<td>1.03 t</td>
<td>1.04 t</td>
<td>1.50 d</td>
<td>1.64(1.65)d</td>
</tr>
</tbody>
</table>

* indicates assignments may be reversed.

$^{130}$ exists as a mixture of diastereomers.
(v) 2-Hydroxygarvin B

In parallel with the garvin A series, the 2-hydroxy derivative of garvin B (130) was isolated as a minor constituent from the *G. annulata* extracts. Purification by LH-20 chromatography and preparative TLC (10% ethyl acetate/CHCl₃) yielded a component which migrated as a single spot on TLC (R₂ 0.35 in standard system). The ¹H NMR spectrum (300MHz, CDCl₃) of 130, however, indicated a 1:1 mixture of diastereomers, since seven of the eleven resonances were doubled at this field strength.

The ¹³C NMR spectrum (see Table 10) also confirmed the presence of a diastereomeric mixture, since fifteen of the twenty one carbon resonances were doubled at a field strength of 75 MHz. The resonances for the alicyclic ring carbons and the three aliphatic methyls were in excellent agreement with the corresponding signals in the spectrum of 2-hydroxygarvin A (112). Similarly, the resonances for the remaining naphthalenic and lactonic substructures of 130 could be matched with those in the ¹³C NMR spectrum of garvin B (126).

We have not determined which of the two chiral centers in 2-hydroxygarvin B is epimeric in the two naturally-occurring diastereomers. However, the fact that garvin B is optically active implies the existence of only one configuration at C16 and suggests that biological hydroxylation at C2 has occurred in non-stereospecific fashion.

Acetylation of 2-hydroxygarvin B with acetic anhydride and pyridine yielded a 1:1 mixture of the epimeric triacetates 131a
130  R = H
131  R = Ac

132  R = H
133  R = Ac
Table 6: $^1$H NMR data for the Acetates of 2-hydroxy derivatives.

Chemical shifts in ppm from TMS. All spectra run in CDCl$_3$.

<table>
<thead>
<tr>
<th>H on C#</th>
<th>97</th>
<th>104</th>
<th>120</th>
<th>131a</th>
<th>131b</th>
</tr>
</thead>
<tbody>
<tr>
<td>20Ac</td>
<td>2.20</td>
<td>2.22</td>
<td>2.25</td>
<td>2.22</td>
<td>2.24</td>
</tr>
<tr>
<td>5</td>
<td>7.61</td>
<td>7.55</td>
<td>7.06</td>
<td>7.50</td>
<td>7.50</td>
</tr>
<tr>
<td>6Or</td>
<td>-</td>
<td>-</td>
<td>3.99</td>
<td>2.39*</td>
<td>2.41*</td>
</tr>
<tr>
<td>80Ac</td>
<td>2.34*</td>
<td>2.45</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>90Ac</td>
<td>2.41*</td>
<td>2.45</td>
<td>2.48</td>
<td>2.45*</td>
<td>2.51*</td>
</tr>
<tr>
<td>10</td>
<td>7.79</td>
<td>7.70</td>
<td>7.73</td>
<td>7.80</td>
<td>7.80</td>
</tr>
<tr>
<td>11</td>
<td>1.45</td>
<td>1.44</td>
<td>1.50</td>
<td>1.47</td>
<td>1.49</td>
</tr>
<tr>
<td>12</td>
<td>1.57</td>
<td>1.55</td>
<td>1.60</td>
<td>1.58</td>
<td>1.63</td>
</tr>
<tr>
<td>13</td>
<td>1.79</td>
<td>1.78</td>
<td>1.83</td>
<td>1.84</td>
<td>1.81</td>
</tr>
<tr>
<td>14</td>
<td>2.41</td>
<td>2.50</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>14OMe</td>
<td>-</td>
<td>-</td>
<td>3.99</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>15</td>
<td>-</td>
<td>2.70 q</td>
<td>2.94 m</td>
<td>3.20 m</td>
<td>3.27 m</td>
</tr>
<tr>
<td>16</td>
<td>2.50</td>
<td>1.13 t</td>
<td>1.70 m</td>
<td>4.56 m</td>
<td>4.58 m</td>
</tr>
<tr>
<td>17</td>
<td>-</td>
<td>-</td>
<td>1.03 d</td>
<td>1.58 d</td>
<td>1.55 d</td>
</tr>
</tbody>
</table>

* indicates assignments may be reversed.
and $131b$ as the major reaction products. It was possible to separate these triacetates by preparative TLC since their $R_f$ values differed by 0.03 in 50% ethyl acetate/hexane. Both $131a$ and $131b$ gave a molecular ion of 510 daltons in the mass spectrum and had almost identical UV spectra. However, their \textsuperscript{1}H NMR spectra showed some distinct differences, especially the chemical shifts of the aliphatic methyl resonances. Both compounds contained three methyl singlets in the 2.2–2.5 ppm region corresponding to the three acetate methyls, and also two aromatic singlets (1H each) at 7.50 and 7.80 ppm.

(vi) **Garvin B Quinone**

Using the same fractionation steps described for the purification of garvin A quinone (122) (LH-20 chromatography, preparative TLC and normal phase HPLC), another bright red compound was purified and identified as garvin B quinone (132). Quinone 132 had a molecular formula of $C_{21}H_{18}O_7$ as determined by HRMS (obt'd. 382.1055, req'd. 382.10xx). The base induced bathochromic shift of the peak at 397 nm (to 463 nm) in the UV spectrum was typical of a naphthoquinone substructure. The \textsuperscript{1}H NMR spectrum (400 MHz, CDCl\textsubscript{3}) contained resonances at 1.58 (s, 3H), 1.60 (s, 3H), 1.95 (s, 3H) and 7.64 (s, 1H) ppm which corresponded closely to those of H11, H12, H13 and H5 in the spectrum of garvin A quinone (122). The six proton spin system [1.63 (d, J=7 Hz, 3H), 3.23 (dd, J=19, 12 Hz, 1H), 3.99 (dd, J=19, 3 Hz, 1H), 4.72 (m, 1H)] indicated the presence of a delta lactone system, similar to that in the spectrum of garvin B (126). One of the methylene protons at C15 has been shifted upfield by 0.36 ppm on
Table 7: $^1$H NMR data (CDCl$_3$) for the quinones.

Chemical shifts in ppm from TMS.

<table>
<thead>
<tr>
<th>H on C#</th>
<th>Garveatin A quinone(105)</th>
<th>Garveatin B quinone(106)</th>
<th>Garvin A quinone(122)</th>
<th>Garvin B quinone(132)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10R</td>
<td>11.66*</td>
<td>11.84*</td>
<td>12.15</td>
<td>12.28*</td>
</tr>
<tr>
<td>5</td>
<td>7.55</td>
<td>7.51</td>
<td>7.56</td>
<td>7.64</td>
</tr>
<tr>
<td>6OR</td>
<td>-</td>
<td>-</td>
<td>4.03*</td>
<td>11.85*</td>
</tr>
<tr>
<td>8OR</td>
<td>11.45*</td>
<td>11.79*</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>11</td>
<td>1.98</td>
<td>1.97</td>
<td>1.98</td>
<td>1.95</td>
</tr>
<tr>
<td>12</td>
<td>1.63</td>
<td>1.61</td>
<td>1.61</td>
<td>1.58*</td>
</tr>
<tr>
<td>13</td>
<td>1.63</td>
<td>1.61</td>
<td>1.61</td>
<td>1.60*</td>
</tr>
<tr>
<td>14</td>
<td>2.41</td>
<td>2.45</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>14OR</td>
<td>-</td>
<td>-</td>
<td>3.98*</td>
<td>-</td>
</tr>
<tr>
<td>15</td>
<td>-</td>
<td>2.78 q</td>
<td>2.95 m</td>
<td>3.23 dd</td>
</tr>
<tr>
<td>16</td>
<td>2.60</td>
<td>1.16 t</td>
<td>1.60 m</td>
<td>3.99 dd</td>
</tr>
<tr>
<td>17</td>
<td>-</td>
<td>-</td>
<td>1.05 t</td>
<td>1.63 d</td>
</tr>
</tbody>
</table>

*,# indicates assignments may be reversed.
replacement of a phenol group with a ketone (3.99 ppm in \textbf{132}, 4.35 ppm in \textbf{126}). This suggests that this proton may be oriented such that it lies in the shielding cone of the C9 ketone in \textbf{132}. Singlets at 11.85 and 12.28 ppm are appropriate for hydrogen-bonded phenol protons at C1 and C6, providing support for the existence of the 3-keto tautomer in chloroform solution, as is also the case for garvin A quinone. The \textsuperscript{13}C NMR spectrum of \textbf{132} (75 MHz, CDCl\textsubscript{3}) is consistent with the proposed structure.

Acetylation of \textbf{132} with acetic anydride/pyridine yielded the diacetate \textbf{133} as a yellow oil. A molecular ion of 466 daltons in the mass spectrum was appropriate for a molecular formula of C\textsubscript{25}H\textsubscript{22}O\textsubscript{9} and a singlet at 2.38 ppm (6H) in the \textsuperscript{1}H NMR spectrum (300 MHz, CDCl\textsubscript{3}) was assigned to the two aromatic acetate methyl groups.

(vii) \textit{Garvin A dimer}

During the purification of garvin A (\textbf{124}) on silica gel, a minor, more non-polar component which had not previously been detected in the crude extracts was characterised and identified as garvin A dimer (\textbf{134}). In parallel with the mass spectra of the dimers of garveatins A and B (\textbf{110} and \textbf{108}), compound \textbf{134} did not give an observable molecular ion in the EI spectrum, the highest-mass peak occurring at 398 daltons corresponding to a monomeric garvin A unit. The \textsuperscript{1}H NMR spectrum of \textbf{134} indicated the presence of the same naphthalenic subunit as in garvin A and 2-hydroxygarvin A. The n-propyl, methyl ether, methyl ester and aromatic protons were all shielded, relative to the corresponding chemical shifts in \textbf{119}. This shielding effect of the naphthalenic
134 \[ R = H \]
135 \[ R = \text{Ac} \]
substituents was also observed in the $^1$H NMR spectra of 108 and 110 and appears to be a feature of 2,2' dimers of this type. Singlets at 1.58, 1.71 and 1.94 ppm (all 3H) were assigned to methyl groups at C2 and C4 (2) of the alicyclic ring and $^{13}$C NMR resonances at 202.5, 63.4, 209.9 and 47.9 ppm were almost identical to those assigned to C1-C4 respectively in the spectrum of garveatin B dimer (108). Therefore the monomeric subunits are connected at the C2 position of 134, similar to the other dimers studied. Irradiation of the methyl singlets at 1.47 and 1.59 ppm induced an nOe in the H10 proton at 7.05 ppm, which assigned these resonances as the gem-dimethyl protons at C12 and C13. Confirmation of the dimeric nature of 134 was obtained by acetylation with acetic anhydride/pyridine to give the diacetate (135). This derivative gave a molecular ion at 878 daltons in the EI mass spectrum corresponding to a molecular formula of $C_{50}H_{54}O_{14}$. Resonances at 2.32 (s,3H) and 7.72 ppm (s,1H(H10)) in the $^1$H NMR spectrum indicated acetylation at the C9 and C9'OH positions.

Subsequently, various reaction conditions were investigated for the conversion of garvin A to the dimer 134. It was found that treatment of a methanolic solution of garvin A with silver oxide under a stream of air produced the dimer 134 in reasonable yield. Furthermore, the dimer could be reconverted to the monomer by hydrogenolysis in the presence of palladium on charcoal, indicating that the 2,2' bond is susceptible to reductive cleavage. However, dimerisation of garvin A did not appear to occur to any significant extent in CDC$_3$ solution as was the case
(viii) Garveatin B-garvin B dimer

Analysis of the less polar fractions obtained by LH-20 chromatography of the G.annulata extracts revealed the presence of a compound with a slightly lower Rf (0.63) than garveatin B dimer (108) in the standard TLC system. Purification by preparative TLC (10% ethyl acetate/CHCl₃) and reverse phase HPLC (95% CH₃CN/H₂O) yielded 7mg of an orange oil whose ¹H NMR spectrum (400MHz,CDC₁₃) indicated sets of resonances corresponding to both garveatin B and garvin B substructures. A FAB mass spectrum showed a molecular ion at 693 daltons (M⁺+1), appropriate for a molecular formula of C₄₁H₄₀O₁₀ and strong fragment ions at m/z 326/327 and 368/369 corresponding to garveatin B and garvin B monomeric units respectively. This data indicated that the compound was a mixed dimer with structure 136. The ¹H NMR spectrum of 136 was consistent with attachment of the monomeric units at the 2,2' position, similar to the other dimers previously characterised. In addition, many of the resonances showed upfield shifts relative to the corresponding resonances in the ¹H NMR spectra of the monomers. In the case of the upfield H15 resonance in 136, the chemical shift (2.35 ppm) differed by more than 1 ppm from that in 2-hydroxygarvin B (3.41 ppm). The phenol proton resonances were assigned on the basis of similarity of two of them (9.10,15.70 ppm) to the phenolic resonances in the spectrum of garveatin B dimer (108).

When equal amounts of garveatin B and garvin B were stirred for 24 hours in a chloroform solution containing silica gel, the
Table 8: $^1$H NMR Data (CDCl$_3$) for the Dimers.

Chemical shifts in ppm from TMS.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Garveatin A dimer(110)</th>
<th>Garveatin B dimer(108)</th>
<th>Garvin A dimer(134)</th>
<th>Garveatin B-Garvin B dimer(136)</th>
</tr>
</thead>
<tbody>
<tr>
<td>H on C#</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>6.98</td>
<td>6.98</td>
<td>6.89</td>
<td>6.90:6.92</td>
</tr>
<tr>
<td>10</td>
<td>7.05</td>
<td>6.98</td>
<td>7.11</td>
<td>6.90:7.02</td>
</tr>
<tr>
<td>11</td>
<td>1.94</td>
<td>1.88</td>
<td>1.94</td>
<td>1.80:1.82*</td>
</tr>
<tr>
<td>12</td>
<td>1.47</td>
<td>1.38</td>
<td>1.58</td>
<td>1.24:1.20*</td>
</tr>
<tr>
<td>13</td>
<td>1.59</td>
<td>1.66</td>
<td>1.71</td>
<td>1.78:1.81*</td>
</tr>
<tr>
<td>14</td>
<td>2.38</td>
<td>2.43</td>
<td>-</td>
<td>2.42: -</td>
</tr>
<tr>
<td>15</td>
<td>-</td>
<td>2.53(m)</td>
<td>2.90(m)</td>
<td>2.42(m):2.35(dd)</td>
</tr>
<tr>
<td>16</td>
<td>2.58</td>
<td>1.07(t)</td>
<td>1.60(m)</td>
<td>1.00(t):4.40(m)</td>
</tr>
<tr>
<td>17</td>
<td>-</td>
<td>-</td>
<td>0.93(t)</td>
<td>- :1.51(d)</td>
</tr>
<tr>
<td>6OR</td>
<td>-</td>
<td>-</td>
<td>3.94</td>
<td>- :11.56</td>
</tr>
<tr>
<td>8OH</td>
<td>10.34</td>
<td>9.37</td>
<td>-</td>
<td>9.10: -</td>
</tr>
<tr>
<td>9OH</td>
<td>15.81</td>
<td>15.79</td>
<td>14.65</td>
<td>15.70:14.85</td>
</tr>
<tr>
<td>14OMe</td>
<td>-</td>
<td>-</td>
<td>3.94</td>
<td>- : -</td>
</tr>
</tbody>
</table>

All resonances are singlets unless indicated otherwise.

* indicates assignment may be reversed.
symmetrical dimer \(108\) and the mixed dimer \(136\) were formed in approximately equal amounts. Therefore, it seems likely that both these compounds are artifacts formed during fractionation of the \textit{G. annulata} extracts. Surprisingly, no evidence of a symmetrical garvin B dimer was detected in the extracts, even though garvin B is one of the most abundant anthracenone metabolites present. This seems to suggest that garveatin B may be the initiator of the dimerisation reaction to produce \(136\), probably via a free-radical mechanism. In view of the fact that garveatin B tautomerises readily in chloroform solution to the diketo form, generation of a free-radical at the C2 position of the diketo tautomer would be a likely initiating step in the reaction sequence.

**Biogenesis**

It appears likely that the same C18 polyketide precursor proposed in the biogenesis of the garveatin series is utilised to form the garvins, via an alternative folding pattern (Scheme 4). Thus, monomethylation and dimethylation of the nonaketide precursor at C8 and C10 respectively, followed by cyclisation could generate an anthracenone containing a 2-oxopropyl group at C7. Reduction of this functionality to an n-propyl group, as well as methylation at the C6 OH and C14 COOH positions yields garvin A (\(124\)). Alternatively, reduction of the 2-oxopropyl group to a secondary alcohol, followed by condensation with a carboxylic acid group at C14 to produce a delta lactone can be envisaged in the biogenesis of garvin B (\(126\)). Further oxidations at C2 and
C9-C10 lead to the 2-hydroxy and the quinone analogs respectively.

\[
\text{Scheme 4.}
\]
Figure 19. 300 MHz $^1$H NMR spectrum of 125 in CDCl$_3$. 

[Diagram of compound 125]
Figure 18a. 400 MHz spin simulation for the n-propyl side-chain of 119.
Figure 19. 300 MHz $^1$H NMR spectrum of 125 in CDCl$_3$. 
Figure 20. 80 MHz $^1$H NMR spectrum of 122 in CDCl$_3$. 
Figure 21. 300 MHz $^1$H NMR spectrum of 129 in CDCl$_3$.  

[Diagram with chemical structure and spectrum data]
Figure 21a. 300 MHz spin simulation for the lactone system of 129.
Figure 22. 270 MHz $^1$H NMR spectrum of 130 in CDCl$_3$. 
Figure 23. 300 MHz $^1$H NMR spectrum of 131a in CDCl$_3$. 
Figure 24. 300 MHz $^1$H NMR spectrum of 131b in CDCl$_3$. 
Figure 25. 270 MHz 1H NMR spectrum of 132 in CDCl₃.
Figure 25a. Comparison of lactone spin system in 400 MHz $^1$H NMR spectrum of 132 with a simulated spectrum.
Figure 26. 400 MHz $^1$H NMR spectrum of 136 in CDCl$_3$. 
Figure 27. 100 MHz $^{13}$C NMR spectrum of 119 in CDCl$_3$. 
Figure 28. 75 MHz $^{13}$C NMR spectrum of 130 in CDCl$_3$. 
Figure 29. 75 MHz $^{13}$C NMR spectrum of 132 in CDCl₃.
C. THE GARVALONES

Closely related to the garvin family are garvalones A (137) and B (141), two minor metabolites which contain the unusual 3-oxobutyl functionality at the C2 position.92

(i) Garvalone A

Garvalone A (137), the more abundant of the two, was detected as a minor component of the LH-20 fractions containing 2-hydroxygarvin A (119). It had a white long-wave UV fluorescence, similar to 119, and was purified by preparative TLC (50% ethyl acetate/hexane) to give 20 mg of a light yellow oil. Compound 137, which was optically inactive, was shown by HRMS to have a molecular formula of C27H32O7 (obs'd 468.2162, req'd 468.2149). A close relationship between the aromatic portion of the previously reported 2-hydroxygarvin A (119) and a fragment of 137 was immediately apparent from a comparison of their NMR and UV data. The UV spectrum (\(\lambda_{\text{max}}\) 228, 279, 317, 330, and 382 nm) of 137 was virtually identical to that of 119 (\(\lambda_{\text{max}}\) 226, 278, 318, 332, and 385 nm) and its \(^1\text{H} \text{NMR} \) spectrum (Table 9) contained resonances that were appropriate for the n-propyl (1.11, t, J=7Hz, 3H; 1.75, m, 2H; 3.15, m, 2H), methyl ester, methyl ether (3.93, s, 3H; 3.96, s, 3H), H5 (6.93, s, 1H) and H10 (7.13, s, 1H) functionalities also found in 119. A nearly exact correspondence of the \(^{13}\text{C} \text{NMR} \) resonances assigned to the carbons in the naphthalene nucleus, the propyl side chain, the methyl ester and methyl ether functionalities in the two compounds...
Functionality which accounted for the remaining atoms (C_{11}H_{16}O_3) of garvalone A (137) could be readily identified upon further consideration of its $^1H$ and $^{13}C$ NMR spectra. Four methyl singlets at 1.47, 1.55, 1.59, and 2.04, and a complex multiplet at 2.23 (4H) ppm located the remaining hydrogen atoms. Carbon resonances at 39.1 (t) and 31.4 (t) required that the protons resonating at 2.23 ppm be attached to two methylene carbons. Carbonyl resonances at 211.9, 207.0, and 203.9 showed that the three oxygens were part of ketone functionalities and resonances at 58.3 (s) and 47.9 (s) ppm identified quarternary carbons.

A comparison of the carbon resonances at 203.9, 58.3, 211.9, and 47.9 ppm in the spectrum of 137 to the resonances exhibited by carbons 1 to 4 in garveatin C (93) (C1, 204.9; C2, 55.3; C3, 211.6; C4, 48.1) showed that they were virtually identical. This correspondence implied that both metabolites had the 1-oxo, 2-dialkyl, 3-oxo, 4-dialkyl functionalities in common. Demonstration of an nOe between the aromatic proton resonance at 7.13(H10) and two methyl resonances at 1.55 and 1.59 ppm in the $^1H$ NMR of 137 provided evidence for a gem-dimethyl group at C4. The $^1H$ NMR resonance at 1.47(s, 3H) was assigned to a methyl substituent at C2 by analogy with the other Garveia metabolites, and a methyl ketone residue was indicated by a $^1H$ NMR resonance at 2.04(s, 3H) and $^{13}C$ NMR resonances at 207.0(s) and 30.5(q) ppm. Insertion of the two methylene carbons between the carbonyl carbon of the methyl ketone residue and the remaining unsatisfied
\[ R = \text{H} \] 
\[ R = \text{CH}_3 \]
valence at C2 of the anthracenone skeleton gave the proposed structure 137 for garvalone A. In the mass spectrum, 137 shows intense fragment ions at 398 (M+ + H - (CH3COCH2CH2-) :100%) and 397 (M+ - (CH3COCH2CH2-) :89%) as would be expected.

Treatment of garvalone A (137) with K2CO3 and methyl iodide in refluxing acetone gave the monomethyl derivative (139) in nearly quantitative yield. The resonances for the four methylene protons, which had appeared as a complex multiplet at 2.23 ppm in the 1H NMR of 137, were more extensively dispersed in the spectrum of 139 (2.48, m, 1H; 2.24, m, 1H; 1.95 to 2.10, m, 2H). A series of decoupling experiments and spin simulations (Figure 31a) showed that these four protons constituted an ABCD spin system, which confirmed the linkage of the two methylene carbons as postulated.

In order to provide a suitable model compound for the cyclohexadione system in 137, a synthesis of 2-(3-oxobutyl) garveatin A (140) was undertaken, utilising a Michael reaction between garveatin A (77) and methyl vinyl ketone93.

Initial attempts to carry out this reaction in the presence of polar solvents such as methanol and anhydrous ethanol (K2CO3, reflux) were unsuccessful and only led to formation of material which remained at the origin when chromatographed on silica-gel. However, using benzene in the presence of K2CO3 and a crystal of 18-crown-6, at reflux under N2 for 24 hours produced the desired product in 80% yield. Apparently, the carbanion (at C2) of garveatin A is more unstable and consequently more reactive in a non-polar solvent such as benzene. The use of crown
ether also appears to facilitate the reaction by chelating the K^+ counter-ion, and thereby enabling the "naked" anion to be alkylated more efficiently.

(ii) Garvalone B

Garvalone B (141), an extremely minor component of the crude extracts, was isolated as an optically active ([α]_D +136.92°, c. 0.39, CHCl_3) light yellow oil. In the mass spectrum, 141 showed a parent ion at m/z 438 appropriate for a molecular formula of C_{26}H_{28}O_6. The ^1H NMR of 141 (table 1) also showed a number of resonances that were doubled, again indicating the presence of two diastereomers (ratio 2:3) which we were unable to separate. The major isomer displayed signals at 1.46(s, 3H), 1.55(s, 3H), 1.57(s, 3H), 2.06(s, 3H) and 2.24(m, 4H) ppm that corresponded to the ^1H NMR resonances displayed by the functionality in the alicyclic ring of garvalone A (137). The remaining ^1H NMR resonances in the spectrum of 141 (1.62(d, J=7HZ, 3H), 3.42(dd, J=12, 18HZ, 1H), 4.39(dd, J=18, 3HZ, 1H), 4.72(m, 1H), 7.06 (s, 1H), 7.10(s, 1H), 11.50(s, 1H) and 15.44(s, 1H)ppm) showed a close correspondence to the observed ^1H NMR resonances for the aromatic and lactonic portions of garvin B (141). Combining the two structural fragments resulted in the proposed structure 141 for garvalone B. Garvalone B was quantitatively converted to the diacetate (142) in accordance with the proposed structure. A synthesis of a mixture of garvalone B diastereomers (141) (ratio 2:3) was achieved via a Michael reaction between garvin B (126) and methyl vinyl ketone. The major isomer formed in the Michael reaction was the minor naturally occurring isomer. Once again,
Table 9: $^1$H NMR Data (CDCl$_3$).

Chemical shifts are in ppm from TMS.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Garvalone A (137)</th>
<th>Garvalone B (141)</th>
<th>2-(3-oxobutyl) garveatin A (140)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbon #</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1OR</td>
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<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2OR</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>6.93</td>
<td>7.06</td>
<td>6.65</td>
</tr>
<tr>
<td>6OR</td>
<td>3.96*</td>
<td>11.50#</td>
<td>-</td>
</tr>
<tr>
<td>8OR</td>
<td>-</td>
<td>-</td>
<td>10.13</td>
</tr>
<tr>
<td>9OR</td>
<td>15.10</td>
<td>15.44#</td>
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<td>1.48</td>
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<tr>
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<td>-</td>
<td>-</td>
</tr>
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<td>1.54#</td>
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<tr>
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<td>2.23,m</td>
<td>2.24,m</td>
<td>2.24 m</td>
</tr>
<tr>
<td>19</td>
<td>2.23,m</td>
<td>2.24,m</td>
<td>2.24 m</td>
</tr>
<tr>
<td>21</td>
<td>2.04</td>
<td>2.06</td>
<td>2.04 m</td>
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*.*, &,# indicate assignments may be reversed.
Table 10: $^{13}$C NMR (CDCl$_3$) data.

Chemical shifts are in ppm from TMS.

<table>
<thead>
<tr>
<th>Compound</th>
<th>2-Hydroxy garvin A(119)</th>
<th>Garvalone A (137)</th>
<th>2-(3-oxobutyl) garveatin A(140)</th>
<th>2-Hydroxy garvin B(130)</th>
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</thead>
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<tr>
<td>Carbon #</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>199.9</td>
<td>203.9</td>
<td>204.1</td>
<td>199.71(199.55)</td>
</tr>
<tr>
<td>2</td>
<td>81.6</td>
<td>58.3</td>
<td>58.0</td>
<td>81.64(81.57)</td>
</tr>
<tr>
<td>3</td>
<td>209.9</td>
<td>211.9</td>
<td>211.3</td>
<td>209.83</td>
</tr>
<tr>
<td>4</td>
<td>46.9</td>
<td>47.9</td>
<td>47.9</td>
<td>47.14(47.05)</td>
</tr>
<tr>
<td>4a</td>
<td>142.9</td>
<td>143.0</td>
<td>142.5*</td>
<td>145.40(145.26)*</td>
</tr>
<tr>
<td>5</td>
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<td>105.0$§$</td>
<td>115.5</td>
<td>111.81</td>
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<tr>
<td>6</td>
<td>157.7</td>
<td>157.4</td>
<td>141.2*</td>
<td>161.27(161.16)</td>
</tr>
<tr>
<td>7</td>
<td>126.9</td>
<td>126.5</td>
<td>125.3</td>
<td>115.94</td>
</tr>
<tr>
<td>8</td>
<td>142.1</td>
<td>142.5</td>
<td>156.0</td>
<td>144.58(144.54)*</td>
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<td>8a</td>
<td>116.7*</td>
<td>116.8*</td>
<td>110.8</td>
<td>110.81(110.77)</td>
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<td>9</td>
<td>166.0</td>
<td>166.6</td>
<td>166.1</td>
<td>167.39(167.17)</td>
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<td>116.8$§$</td>
<td>120.2</td>
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<tr>
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<td>108.7*</td>
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<td>29.15(29.04)#</td>
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<td>-</td>
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<td>27.1#</td>
<td>30.70(30.44)#</td>
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<td>168.3</td>
<td>168.6</td>
<td>20.7</td>
<td>169.85(169.80)</td>
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<td>36.0</td>
<td>36.1</td>
<td>204.2</td>
<td>34.60(34.36)</td>
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<td>16</td>
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<td>25.4</td>
<td>32.3</td>
<td>75.43(75.35)</td>
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<td>-</td>
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<td>31.3</td>
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<td>38.9</td>
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<th></th>
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<th>206.9</th>
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<td>30.5</td>
<td>30.7</td>
<td>-</td>
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<td>60R</td>
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<td>55.9</td>
<td>-</td>
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<tr>
<td>140R</td>
<td></td>
<td>52.3</td>
<td>52.5</td>
<td>-</td>
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</table>

*,# indicate that the assignments may be interchanged.

![Chemical Structure]

141 \( R = H \)
142 \( R = Ac \)
since garvin B occurs as a single enantiomer, it seems reasonable
to assume that it is the chiral center at C2 which is epimeric in
the natural garvalone B stereoisomers. This assignment is
consistent with the lack of optical activity found for garvalone
A (137) which implies a nonstereospecific alkylation at C2 in
both molecules. The possibility that the garvalones are artifacts
due to contamination of solvents by methyl vinyl ketone was ruled
out by the following evidence: (a) the garvalones were found
repeatedly in _G. annulata_ collections; (b) if methyl vinyl ketone
was present in any of the solvents used in the extraction and
purification procedures, it would be reasonable to expect that
the major alkylation product would be 2-(3-oxobutyl) garveatin A
(140), since garveatin A is by far the most abundant polyketide
metabolite. Compound 140 has never been detected in any of the _G.
annulata_ collections to date, even though it can be readily
synthesised via the Michael reaction discussed previously.

**Biogenesis:**

The structural similarity of the garvalones to the garvins
suggests a common biogenetic origin from a nonaketide precursor
as outlined in the previous chapter. Alkylation at the C10
position of the precursor (either before or after cyclisation) by
an activated four-carbon moiety such as an acetoacetyl derivative
(Scheme 5), followed by reduction of one of the ketone groups
seems to be a plausible pathway for the biosynthesis of the 2-(3-
oxobutyl) side-chain of the garvalones. The obvious lack of
stereospecificity of this alkylation raises serious doubts as to
whether this step is subject to enzymatic control. A possible explanation may be that garvins A and B are acting as free-radical or electrophilic scavengers in vivo by reacting with a four-carbon compound such as a methyl vinyl ketonyl or acetoacetyl moiety via a Michael addition at C2. Similarly, the 2-hydroxygarvin derivatives may have been formed from the garvins by free-radical attack of molecular oxygen at C2, suggesting an antioxidant role for these compounds.
Figure 30. 400 MHz $^1$H NMR spectrum of 137 in CDCl$_3$. 
Figure 31. 400 MHz 1H NMR spectrum of 132 in CDCl₃.
Figure 31a. Spin simulation of H18-19 system in 400 MHz spectrum of 139.
Figure 32. 400 MHz $^1$H NMR spectrum of 140 in CDCl$_3$. 
Figure 33. 80 MHz $^1$H NMR spectrum of 141 in CDCl$_3$. 
Figure 34. 75 MHz $^{13}C$ NMR spectrum of 137 in CDCl$_3$. 
Figure 35. 75 MHz $^{13}$C NMR spectrum of 140 in CDCl$_3$. 
D. THE ANNULINS.

The fourth and final class of metabolites obtained from *G. annulata*, called the annulins, are characterised by a degraded anthracene skeleton in which the alicyclic six-membered ring has been cleaved.

TLC analysis of the flash fraction eluted with 50% ethyl acetate/hexane indicated the presence of two unidentified compounds with Rf's close to 0.5 in the standard system, and which gave a positive color reaction with I2 vapour. This fraction (500 mg) was chromatographed on LH-20 (90% MeOH/CH2Cl2) to give a major peak which was further purified by preparative TLC and normal phase HPLC to yield two pure compounds, annulin A (144) (20 mg) and annulin B (148) (12 mg). When spotted on a TLC plate and subjected to the vapours of a 25% aqueous NH3 solution, annulin A went dark purple while annulin B turned bright pink. This colour reaction indicated the presence of a quinone functionality in these molecules, and it is likely that the NH3 reacts with the carbonyl group of the quinone to form an imine.

(i) Annulin A

Annulin A (144), obtained from 95% ethanol as bright orange crystals, was shown by HRMS to have a molecular formula of C19H20O7 (M+ 360.1221, req'd 360.1209). The 1H NMR spectrum of annulin A contained resonances which could be assigned to aromatic methyl (2.44, s, 3H), and ethyl (1.14, t, 3H; 2.76, q,
2H) substituents, a methyl ether or ester (3.87, s, 3H), and two aliphatic methyl groups (1.63, s, 3H; 1.72, s, 3H). Subtracting the six carbon atoms required by the methyl and ethyl resonances observed in the $^1$H NMR of annulin A from its molecular formula leaves a residue of thirteen carbons, indicating that the fourteen carbon anthracene-type skeleton of the previously reported Garveia metabolites could not be present.

Additional resonances in the $^1$H NMR spectrum of annulin A could be assigned to a single aromatic proton (7.47, s, 1H) and a phenolic proton (12.12, s, 1H). The chemical shifts of the resonances assigned to the aromatic proton, the phenolic proton, and the aromatic methyl and ethyl groups bore a striking resemblance to the chemical shifts of the corresponding resonances in the $^1$H NMR spectrum of garveatin B quinone (106), an artifact formed during silica gel purification of garveatin B (88). This similarity (see Table 11) suggested that annulin A contained a napthaquinone nucleus with substituents on the aromatic ring which were identical to those present in garveatin B quinone (106). Consistent with this assignment was the observation of H-bonded (1616 cm$^{-1}$) and non H-bonded (1657 cm$^{-1}$) quinone carbonyl stretching bands in the IR, a base induced bathochromic shift to 559nm characteristic of napthaquinones in the UV, and two carbonyl resonances at 180.91 and 185.97 ppm in the $^{13}$C NMR spectrum of annulin A (table 12).

The remaining functionality in annulin A could be readily identified from its spectral data. $^{13}$C NMR resonances at 169.35 and 54.04, in conjunction with the $^1$H NMR resonance at 3.87 ppm
and an IR band at 1750 cm\(^{-1}\), indicated a methyl ester. A \(^{13}\)C NMR resonance at 101.45 and a \(^{1}\)H NMR resonance at 4.92 (bs, 1H) were assigned to a hemiketal, while a \(^{13}\)C NMR resonance at 89.08 ppm was assigned to a tertiary ether carbon. Four candidate structures (144-147) could be constructed from the identified fragments.

The expected equilibration of the hemiketal functionality in structures 145 and 147 would give in both cases a mixture of two diastereomers which would be expected to have quite different \(^1\)H NMR spectra. Careful examination of the \(^1\)H NMR spectrum of annulin A failed to uncover any evidence for a mixture of diastereomers, suggesting that structures 145 and 147 were improbable candidates. Equilibration of the hemiketal functionality in structures 144 and 146 would effect racemization. Our initial attempt to measure the optical activity of annulin A was positive ([\(\alpha\)]\(_D\) +24.9\(^\circ\);c 0.35), casting doubt on the validity of candidate structures 144 and 146. A limited supply of annulin A (<4mg) precluded a spectroscopic or chemical resolution of this dilemma. The structure of annulin A (144) was solved by single-crystal x-ray diffraction analysis by Xu and Clardy at Cornell.

A computer generated drawing of the final x-ray model of annulin A (144) is given in Figure 36. Annulin A is a naturally occurring racemate, and the enantiomer shown is arbitrary. The only chiral center, Cl, is a hemiketal which presumably epimerizes as argued above. The five-membered ring is essentially planar; all internal torsional angles are less than 10\(^\circ\). Thus the tricyclic portion of annulin A is planar within experimental error. There appear to be intramolecular hydrogen bonds between
Figure 36. Computer generated X-ray structure of Annulin A (144).
H05. As expected, the ethyl sidechain is rotated out of the molecular plane by roughly 90°.

A re-examination of the optical activity of annulin A (144), using a larger sample obtained from a second isolation, demonstrated that our initial observation of optical activity was erroneous.

(ii) **Annulin B**

Annulin B (148) was obtained as an optically active \([\alpha]_D^{+8.0; c 0.2}\) orange oil that was shown by HRMS to have a molecular formula of \(C_{21}H_{22}O_7\) (\(M^+ 386.1361\), req'd 386.1366). The \(^1\text{H} \text{NMR}\) spectrum of annulin B again revealed the presence of a napthaquinone substructure containing hydroxyl, ethyl, and methyl substituents on the aromatic ring as in annulin A (144) (Table 11). Support for this fragment came from IR bands at 1657 and 1638 cm\(^{-1}\) assigned to the quinone carbonyl stretching vibrations, a base induced bathochromic shift to 530nm in the UV, and \(^13\text{C} \text{NMR}\) resonances at 181.14 and 178.62 ppm assigned to the quinone carbonyl carbons.

Functionality accounting for the remaining atoms in annulin B (\(C_{8}H_{12}O_4\)) was identified from its spectral data. \(^13\text{C} \text{NMR}\) resonances at 167.68 and 53.45, a \(^1\text{H} \text{NMR}\) resonance at 3.76 ppm (s, 3H), and an IR band at 1757 cm\(^{-1}\) identified a methyl ester. \(^1\text{H} \text{NMR}\) resonances at 1.49 (s, 3H), 1.51 (s, 3H), and 1.85 (s, 3H) ppm could be assigned to three aliphatic methyl groups and a gated decoupled \(^13\text{C} \text{NMR}\) spectrum of 148 contained resonances at 23.78 and 25.90 ppm that both appeared as quartets of quartets typical of a gem-dimethyl moiety. An IR band at 1738 cm\(^{-1}\) and a
The presumption of a common biogenesis for annulin B (148) and the rest of the Garveia metabolites led us to situate the ketone and the gem-dimethyl array at positions corresponding to C3 and C4 in the anthracene skeleton of the other metabolites. The chemical shift of the quarternary carbon resonance in the spectrum of 148 (43.64 ppm), which was close to that assigned to C4 (46.9 ppm) in 2-hydroxygarvin A (119), supported this placement. The chemical shift of the tertiary carbon (84.48 ppm) in the spectrum of annulin B was quite similar to the chemical shift of the C2 carbon in 119 (81.6 ppm), implying that it too was attached to an oxygen, a single alkyl, and two carbonyl carbons. Thus the tertiary carbon in annulin B had to be attached to the ketone carbonyl at C3, the ester carbonyl, the remaining aliphatic methyl, and the remaining oxygen atom. The final site of unsaturation required by the molecular formula of annulin B could be generated by forming an ether linkage between the oxygen atom on C2 and an unsatisfied valence at C9a resulting in the proposed structure 148 for annulin B.

A complete $^{13}$C NMR assignment for annulin B is given in Table 12. The assignments were made from empirical calculations using juglone$^{75}$ and rugulosin$^{95}$ as model compounds. Of particular note are the resonances at 163.8 and 119.1 ppm assigned to C9a and C4a respectively which reflect the influence of the ether oxygen on the olefinic carbons of the quinone. Thus,
### Table 11: $^1$H NMR (CDCl$_3$) data.

Chemical shifts are in ppm from TMS.

<table>
<thead>
<tr>
<th>Proton on Carbon no.</th>
<th>Annulin A (144)</th>
<th>Annulin B (148)</th>
<th>Garveatin B quinone (106)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-OH</td>
<td>4.92s</td>
<td></td>
<td>11.79s</td>
</tr>
<tr>
<td>3</td>
<td>3.87s</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>7.47s</td>
<td>7.31s</td>
<td>7.51s</td>
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<td>12.12s</td>
<td>12.35s</td>
<td>11.84s</td>
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<td>1.85s</td>
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</tr>
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<td>1.63s*</td>
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<td>1.61s</td>
</tr>
<tr>
<td>13</td>
<td>1.72s*</td>
<td>1.51s*</td>
<td>1.61s</td>
</tr>
<tr>
<td>14</td>
<td>2.44s</td>
<td>2.42s</td>
<td>2.45s</td>
</tr>
<tr>
<td>15</td>
<td>2.76q</td>
<td>2.73q</td>
<td>2.78q</td>
</tr>
<tr>
<td>16</td>
<td>1.14t</td>
<td>1.15t</td>
<td>1.16t</td>
</tr>
<tr>
<td>17</td>
<td></td>
<td>3.76s</td>
<td></td>
</tr>
</tbody>
</table>

* assignments may be reversed.
Table 12: $^{13}$C NMR (75 MHz, CDCl$_3$) data.

Chemical shifts are in ppm from TMS.

<table>
<thead>
<tr>
<th>Carbon ns.</th>
<th>Annulin A(144)</th>
<th>Annulin B(148)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>101.45</td>
<td>167.68</td>
</tr>
<tr>
<td>2</td>
<td>169.35</td>
<td>84.48</td>
</tr>
<tr>
<td>3</td>
<td>54.04</td>
<td>203.02</td>
</tr>
<tr>
<td>4</td>
<td>89.08</td>
<td>43.64</td>
</tr>
<tr>
<td>4a</td>
<td>154.29</td>
<td>119.11</td>
</tr>
<tr>
<td>5</td>
<td>122.10</td>
<td>120.65</td>
</tr>
<tr>
<td>6</td>
<td>145.63</td>
<td>147.64</td>
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<tr>
<td>7</td>
<td>139.81*</td>
<td>136.06</td>
</tr>
<tr>
<td>8</td>
<td>160.36</td>
<td>160.43</td>
</tr>
<tr>
<td>8a</td>
<td>113.40</td>
<td>111.11</td>
</tr>
<tr>
<td>9</td>
<td>185.97</td>
<td>181.14</td>
</tr>
<tr>
<td>9a</td>
<td>140.76*</td>
<td>163.79</td>
</tr>
<tr>
<td>10</td>
<td>180.91</td>
<td>178.62</td>
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<tr>
<td>10a</td>
<td>130.37</td>
<td>127.59</td>
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<tr>
<td>11</td>
<td></td>
<td>20.38</td>
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<tr>
<td>12</td>
<td>26.43$</td>
<td>23.78*</td>
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<tr>
<td>13</td>
<td>27.94$</td>
<td>25.90*</td>
</tr>
<tr>
<td>14</td>
<td>20.08</td>
<td>20.40</td>
</tr>
<tr>
<td>15</td>
<td>19.50</td>
<td>19.10</td>
</tr>
<tr>
<td>16</td>
<td>12.73</td>
<td>12.77</td>
</tr>
<tr>
<td>17</td>
<td></td>
<td>53.45</td>
</tr>
</tbody>
</table>

*,: assignments may be reversed.
C9a is strongly deshielded by the electron withdrawing effect of the oxygen atom, whereas C4a experiences a shielding effect due to a resonance contribution arising from delocalisation of the lone-pair on the ether oxygen atom. A similar effect is seen in the $^{13}$C NMR spectrum of stemphone (149), a pigment from the fungal pathogen *Stemphylium sarcinaeforme*. This compound also contains a six-membered ether ring fused to a quinone, and resonances at 151.6 and 119.2 ppm for C5 and C6 respectively are comparable to those of C9a and C4a in annulin B.

**Biogenesis:**

Annulins A (144) and B (148) both appear to be degradation products of garveatins. The conversion of garveatin B (88) to annulin B (148) requires oxidation of the central ring to a quinone, hydroxylation at C2, cleavage of the C1-C9a bond, and oxidation of the C1 carbon to a carboxylic acid. 2-Hydroxygarveatin B (102), a co-occurring metabolite, is a possible intermediate in this pathway. Conversion of any potential garveatin precursor to annulin A (144) requires the removal of at least one carbon atom (C3) in addition to oxidation state transformations (see Scheme 6).

Somewhat surprisingly, no corresponding degraded analogs of garveatin A were detected in the extracts of *G. annulata*. In view of the fact that garveatin A is present in much higher concentration than garveatin B, it seems unlikely that garveatin A and its derivatives are metabolised to annulin-type products.
Scheme 6.
Figure 37. 300 MHz $^1$H NMR spectrum of 144 in CDCl$_3$. 
Figure 38. 80 MHz $^1$H NMR spectrum of 148 in CDCl$_3$. 
Figure 39. 75 MHz 13C NMR spectrum of 144 in CDCl₃.
Figure 40. 75 MHz $^{13}$C NMR spectrum of 148 in CDCl$_3$. 
E. CONCLUSION.

A variety of polyketide-derived secondary metabolites have been obtained from the methanol extracts of the marine hydroid *Garveia annulata*, collected off Vancouver Island, B.C. These have been divided into four distinct groups based on their carbon skeletons: the garveatins, the garvins, the garvalones and the annulins.

The garveatins and garvins both contain oxidized metabolites in the form of 2-hydroxy analogs, 9,10 quinones and 2,2′ dimers. The observance of diastereomeric forms of 2-hydroxy garvin B in a 1:1 ratio, combined with the lack of optical activity in any of the other 2-hydroxy derivatives tested, suggests a non-enzymatic route for conversion of the parent keto-enol compounds to the corresponding 2-hydroxy derivatives. Likewise, the quinones and dimers appear to be produced by aerial oxidation processes. However, the presence of considerable amounts of the 2-hydroxy compounds and quinones in the fresh extracts indicates that these compounds may well be present in the intact organism. The low yields of oxidation products obtained on vigorous oxidation of garveatin A in the laboratory tends to support this hypothesis.

The apparent lack of chirality at the C2 center in the garvalones also casts serious doubts as to whether the insertion of the 3-oxobutyl group is subject to biological control. The fact that garvalone A has been found repeatedly in different collections of *G. annulata* precludes its formation from garvin A.
by contamination of the solvents with methyl vinyl ketone. Addition of 1 ml of methyl vinyl ketone to a 4 litre jar containing a fresh methanol extract of *G. annulata* did not produce any noticeable increase in the yield of garvalone A on subsequent work-up. This evidence seems to indicate that the garvalones are present in the intact organism and are derived from the parent garvins A and B by a non-stereospecific addition of a C4 unit to the C2 position.

The *annulins* represent degraded forms of garveatin B where the central ring has been converted to a quinone moiety and the alicyclic ring has been subjected to oxidative cleavage. It is not apparent why the corresponding degraded analogs of the much more abundant garveatin A have not been detected in the extracts.

The *G. annulata* metabolites exhibit considerable antibiotic activity as seen in the results of the *in-vitro* antibacterial and antifungal assays (Table 13). Minimum inhibitory concentrations of 1 ug or less were recorded for some of the compounds tested in the antibacterial assays. In general, these metabolites appear to be more toxic to bacteria than to fungi.

It is interesting to note that no antibiotic activity was detected in the crude aqueous extracts of *G. annulata*, neither was there a noticeable orange color characteristic of the polyketides. It had been anticipated that some of the hydroid metabolites might be present as water-soluble sulfate esters, analogous to those reported by Sutherland\(^54,62\) from various species of crinoids. However, no anthracenone-type compounds were detected in the aqueous extracts. The crinoidal sulfate esters\(^57\)
Table 13. Results of *in-vitro* antibiotic assays for the *G. annulata* metabolites. Minimum inhibitory concentrations reported in ug/disc.

<table>
<thead>
<tr>
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<th></th>
<th></th>
<th></th>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td>Garveatin A</td>
<td>7</td>
<td>20</td>
<td>20</td>
<td>7</td>
<td>nt.</td>
</tr>
<tr>
<td>Garveatin B</td>
<td>10</td>
<td>10</td>
<td>80</td>
<td>80</td>
<td>80</td>
</tr>
<tr>
<td>Garveatin C</td>
<td>30</td>
<td>30</td>
<td>na.</td>
<td>30</td>
<td>nt.</td>
</tr>
<tr>
<td>Garveatin D</td>
<td>2.5</td>
<td>2.5</td>
<td>na.</td>
<td>na.</td>
<td>nt.</td>
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<tr>
<td>2-hydroxy garveatin A</td>
<td>372</td>
<td>31</td>
<td>124</td>
<td>31</td>
<td>nt.</td>
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<tr>
<td>2-hydroxy garveatin B</td>
<td>13</td>
<td>13</td>
<td>na.</td>
<td>64</td>
<td>na.</td>
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<tr>
<td>Garveatin A quinone</td>
<td>1</td>
<td>4</td>
<td>50</td>
<td>50</td>
<td>nt.</td>
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<td>Garveatin B quinone</td>
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<td>10</td>
<td>40</td>
<td>40</td>
<td>126</td>
</tr>
<tr>
<td>Garvin A</td>
<td>20</td>
<td>20</td>
<td>80</td>
<td>80</td>
<td>nt.</td>
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<td>2-hydroxy garvin A</td>
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<td>109</td>
<td>190</td>
<td>430</td>
<td>1300</td>
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<tr>
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<td>2</td>
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<tr>
<td>Garvin B</td>
<td>30</td>
<td>4</td>
<td>na.</td>
<td>160</td>
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</table>
Table 13, cont'd.

<table>
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</thead>
<tbody>
<tr>
<td>2-hydroxy garvin B</td>
<td>29</td>
<td>29</td>
<td>348</td>
<td>348</td>
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<tr>
<td>Garvin B quinone</td>
<td>10</td>
<td>10</td>
<td>na.</td>
<td>na.</td>
<td>nt.</td>
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<td>Garvalone A</td>
<td>85</td>
<td>255</td>
<td>na.</td>
<td>na.</td>
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<tr>
<td>Garvalone B</td>
<td>60</td>
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<td>60</td>
<td>180</td>
<td>nt.</td>
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<td>Annulin A</td>
<td>2</td>
<td>0.5</td>
<td>40</td>
<td>40</td>
<td>80</td>
</tr>
<tr>
<td>Annulin B</td>
<td>1.5</td>
<td>7.5</td>
<td>na.</td>
<td>na.</td>
<td>nt.</td>
</tr>
</tbody>
</table>

**Bacteria:**

Bc.: Bacillus subtilis. St.: Staphylococcus aureus.

**Fungi:**

Helm.: Helminthosporium sativum.
na.: not active at maximum concentration tested.
nt.: not tested.
were shown to have strong fish anti-feedant activity when tested on a number of local marine species at the concentrations found in the crinoids. A similar type of fish-antifeedant bioassay was conducted in this laboratory using goldfish as the test species and employing food pellets containing varying amounts of garveatin A (22) up to 500 µg. The fish did not discriminate between test and control pellets, suggesting that the *G. annulata* polyketides probably don't play a defensive role against fish predators in the marine environment.

The 1-anthracenone skeleton elaborated by many of the *G. annulata* polyketides is typical of several potent antibiotic and antineoplastic drugs such as mithramycin\textsuperscript{97}, the chromomycins\textsuperscript{98} and the olivomcins\textsuperscript{99}, isolated from various fungal species. They also share some structural similarities with the anthracyclines daunomycin and adriamycin produced by a *Streptomyces* species\textsuperscript{100}. All of the above fungal metabolites contain sugar residue(s) linked to the aglycone portion. In contrast, no evidence of carbohydrate-containing polyketides has been found in the aqueous or organic phases of *G. annulata*. The only compound characterised from the aqueous phase of the *G. annulata* extracts was homarine (N-methylpicolinic acid) (150), a metabolite which has previously been found in several marine invertebrates\textsuperscript{101}. This substance proved to be the most abundant low molecular weight component and was characterised by comparison of its \textsuperscript{1}H and \textsuperscript{13}C NMR data with those obtained from another source\textsuperscript{102}. There is evidence\textsuperscript{103} that homarine acts as a transmethylating agent in shrimp muscle homogenates and is
capable of transferring its methyl group to various acceptor molecules. Netherton and Gurin speculate that homarine is not only a methyl donor (analogous to S-adenosyl methionine) but may serve as a reservoir of methyl groups in crustacea. This raises the intriguing possibility that homarine may be involved in the methylation of the *G. annulata* polyketides.

The true metabolic role of the polyketide metabolites in the life-cycle of *G. annulata* remains a mystery. Apart from their potent *in-vitro* antibiotic activity which points to a possible defensive role against marine micro-organisms, the demonstrated reactivity of the keto-enol compounds suggests that they might act as anti-oxidants or free-radical scavengers *in-vivo*. Considering the high concentrations of polyketides present in the hydroid, they would be expected to serve a distinct biological role since the organism obviously expends a large amount of metabolic energy in synthesising them. These compounds appear to be endogenous since there is no evidence of a symbiotic relationship with any microbial species, and no indication that they might be derived from a dietary source.
III: EXPERIMENTAL

The $^1$H NMR spectra were recorded on Bruker WP-80, Nicolet-Oxford 270, Varian XL-300 and Bruker WH-400 spectrometers. $^{13}$C NMR spectra were recorded on Bruker WP-80, Bruker WH-400 and Varian XL-300 spectrometers. TMS was used as an internal standard. Low resolution mass spectra were recorded on an AEI MS902 spectrometer and high resolution mass spectra on an AEI MS50 instrument. IR spectra were recorded on BOMEM Fourier Transform and Perkin-Elmer 710B spectrometers. UV-Visible spectra were recorded on a Bausch and Lomb Spectronic-2000 spectrometer. Optical rotation measurements were recorded on a Perkin-Elmer 141 polarimeter using a 10cm microcell. A Fisher-Johns apparatus was used to determine melting points and these values are uncorrected.

Merck Silica gel 230-400 mesh was used for flash and preparative thin layer chromatography and Whatman Magnum-9 Partisil-10 and Partisil-10 ODS 3 columns were used for preparative HPLC. Sephadex LH-20 resin was used for molecular exclusion chromatography. $R_f$'s are listed for all compounds in an analytical TLC system using a 1:50:50 acetic acid/ethyl acetate/hexane eluent. The HPLC solvents were Fisher grade or BDH HPLC grade. Water was glass-distilled and all other solvents were reagent grade.
Collection Data

Garveia annulata was collected by hand using SCUBA (-2 to -9m) on exposed rocky reefs in Barkley Sound, British Columbia during the winter and spring months.

Extraction and Chromatography

Freshly collected whole specimens were immediately placed in methanol and stored at room temperature. The methanol extract was decanted and filtered after three days. The filtrate was rotary-evaporated to give an aqueous suspension that was diluted to 400ml with distilled water and extracted successively with hexane(3x400ml), methylene chloride(3x400ml) and ethyl acetate(2x400ml).

The hexane(600mg), methylene chloride(1.5g) and ethyl acetate(700mg) phases were fractionated separately by step-gradient vacuum flash chromatography using a 3.5cm thick silica pad in a sintered-glass funnel (10cm diameter). Fractions eluting with the same solvent composition from each separation were combined. Elution with 20% ethyl acetate/hexane, 50% ethyl acetate/hexane, 100% ethyl acetate, and 20% methanol/ethyl acetate gave fractions A(140mg), B(500mg), C(1.5g) and D(700mg) respectively.

Fraction A:

Purification of fraction A on a Sephadex LH-20 column (3.5cm dia. x 900cm) using 90% MeOH/CH₂Cl₂ as the eluant gave subfractions (i) and (ii) in order of elution.
Fractionation of (i) by preparative TLC (40% ethyl acetate/hexane) gave garveatin C (93) (20mg).

Purification of (ii) by preparative TLC (10% ethyl acetate/hexane) and reverse-phase HPLC (95% CH$_3$CN/H$_2$O) yielded garveatin B–garvin B dimer (136) (6mg).

**Fraction B:**

Purification of fraction B by LH-20 chromatography (90% MeOH/CH$_2$Cl$_2$) gave subfractions (iii) to (viii), in order of elution.

Subfraction (iii) was purified by preparative TLC (50% ethyl acetate/hexane) to give garvalone A (137) (20mg).

Subfraction (iv) was purified by preparative TLC (10% ethyl acetate/CHCl$_3$) to yield 2-hydroxygarvin A (119) (100mg).

Subfraction (v) was fractionated by preparative TLC (50% ethyl acetate/hexane) to give impure annulin A and pure annulin B (148) (12mg). Chromatography on normal phase HPLC (40% ethyl acetate/hexane) gave pure annulin B (144) (12mg). When spotted on TLC plates and exposed to the vapours of a 25%aq. NH$_3$ solution, annulin A turned dark purple while annulin B gave a bright pink spot.

Subfraction (vi) had a deep red color and was purified by preparative TLC (50% ethyl acetate/hexane) and normal phase HPLC (ethyl acetate/hexane gradient) to give garvin A quinone (122) (10mg) and garvin B quinone (132) (8mg).

Subfraction (vii) was purified by preparative TLC (25% ethyl acetate/hexane) to yield garveatin B dimer (108) (3mg) and
impure garveatin B. The latter was purified on normal phase HPLC (ethyl acetate/ hexane gradient) to give garveatin B (88) (15mg).

Subfraction (viii) was purified by preparative TLC (10% ethyl acetate/ hexane) to yield 2-hydroxygarvin B (130) (14mg) as a mixture of diastereomers.

**Fraction C:**

Purification of fraction C by LH-20 chromatography (90% MeOH/CH$_2$Cl$_2$) yielded subfractions (ix) to (xiv), in order of elution.

Subfraction (ix) was purified by preparative TLC (50% ethyl acetate/ hexane) to give 2-hydroxy garveatin B (102) (40mg).

Subfraction (x) was purified by preparative TLC (10% ethyl acetate/ CHCl$_3$) to yield garvin A dimer (134) (5mg) and garvin A (124) (35mg).

Subfraction (xi) was purified by preparative TLC (5% MeOH/CHCl$_3$ and 25% ethyl acetate/ hexane) to give garvalone B (141) (3mg) and garveatin D (96) (8mg).

Subfraction (xii) was purified by preparative TLC (2% MeOH/ CHCl$_3$) to give garveatin A quinone (105) (11mg).

Subfraction (xiii) eluted late as a very broad yellow band and contained almost pure garveatin A. Final purification by trituration with 80% CHCl$_3$/ hexane and crystallisation from acetone gave pure garveatin A (77) (300mg).

Subfraction (xiv) contained garvin B mixed with a small amount of garveatin A. The latter was removed by trituration with acetone to give pure garvin B (126) (100mg).
Fraction D:

Fraction D contained a mixture of relatively polar compounds which were applied on LH-20 (90% MeOH/CH$_2$Cl$_2$) to give subfraction (xv). Purification of a portion of (xv) by reversed phase preparative TLC (80% MeOH/H$_2$O) gave 2-hydroxy garveatin A (97) (12mg).
Spectral Data:

Garveatin A (77): obtained as yellow-orange needles; m.p. 236-240°C (cryst. from acetone); UV (MeOH) neutral/basic 232 (18000), 282 (10000), 323(sh) (5000), 432 (12000), acidic 238 (17000), 253(sh) (12000), 298 (7000), 402 nm (7000); IR (CHCl₃) 3010, 1725 (sh), 1680, 1610 cm⁻¹; ¹H NMR (80 MHz,CDCl₃) 1.63 (s,6H), 1.98 (s,3H), 2.40 (br s,3H), 2.69 (s,3H), 7.02 (br s,1H), 7.15 (s,1H), 10.56 (s,1H), 17.34 ppm (s,1H); ¹H NMR (300MHz,acetone d₆) 1.58 (s,6H), 1.99 (s,3H), 2.41 (br s,3H), 2.70 (s,3H), 6.87 (br s,1H), 6.97 ppm (s,1H); ¹³C NMR (75 MHz,acetone d₆) 204.12 (s), 198.64 (s), 175.89 (s), 170.47 (s), 161.63 (s), 149.09 (s), 138.53 (s), 136.88 (s), 120.61 (s), 118.32 (d), 113.94 (s), 110.09 (d), 105.95 (s), 103.25 (s), 45.36 (s), 32.69 (q,2C), 30.17 (q), 21.28 (q), 7.70 (q) ppm; HRMS 340.1317, calc'd for C₂₂H₂₂O₅ 340.1311; LRMS m/z (rel. intensity) 340 (19), 325 (38), 310 (5), 283 (5).

Preparation of 3-methyl garveatin A (78):

Garveatin A (77) (16mg) was dissolved in diethyl ether (3 ml, anhyd.) and treated with diazomethane (generated by adding 0.6 ml 5M NaOH to N-methylnitro-nitroso-guanidine). After standing for 3 hours the reaction mixture was purified by preparative chromatography (10% ethyl acetate/CHCl₃) to yield 3-methyl garveatin A (78) (3 mg) as the major reaction product.

Compound (78): obtained as an orange oil. Rₚ 0.54; UV (MeOH) neutral 238, 286, 405 nm, basic 238, 286, 429 nm; ¹H NMR (80 MHz,CDCl₃) 1.58 (s,6H), 2.08 (s,3H), 2.41 (br s,3H), 2.66 (s,3H), 4.04 (s,3H), 7.01 (br s,1H), 7.13 (s,1H), 10.40 (s,1H), 17.25 ppm
Preparation of Dimethyl (79) and Trimethyl Garveatin A (80):

A suspension of 37 mg of garveatin A (77), 550 mg K$_2$CO$_3$ and 400 ul (4.2 mmoles) of dimethyl sulfate in 3 ml acetone was heated at reflux under an N$_2$ atmosphere for 21 hours. After cooling, 4 ml of H$_2$O and 400 ul 1M KOH were added. After two hours the mixture was extracted with CH$_2$Cl$_2$ (4x4ml). The aqueous phase was acidified to pH 2 and extracted with 2x5ml CH$_2$Cl$_2$. The CH$_2$Cl$_2$ extracts were dried over Na$_2$SO$_4$ and concentrated. Preparative chromatography using 5% ethyl acetate/hexane and 1% acetic acid/CH$_2$Cl$_2$ yielded dimethyl garveatin A (79) (6 mg) and trimethyl garveatin A (80) (15 mg). Compound (79): obtained as a yellow oil. $R_f$ 0.53; UV (MeOH) 206, 235, 286, 494 nm; $^1$H NMR (80 MHz,CDCl$_3$) 1.59 (s,3H), 2.08 (s,3H), 2.38 (br s,3H), 2.63 (s,3H), 3.91 (s,3H), 4.01 (s,3H), 7.20 (s,1H), 7.30 (br s,1H), 15.83 ppm (s,1H); HRMS 368.1625, calc'd for C$_{22}$H$_{24}$O$_5$ 368.1624; LRMS m/z (rel. intensity) 368 (93), 353 (100), 337 (18). Compound (80): obtained as a yellow-orange oil. $R_f$ 0.43; UV (MeOH) 230, 265, 306 (sh), 360 nm; IR (CHCl$_3$) 3600, 3000, 2410, 1690, 1620, 1425, 1320, 1210 cm$^{-1}$; $^1$H NMR (80 MHz,CDCl$_3$) 1.63 (s,6H), 2.08 (s,3H), 2.38 (br s,3H), 2.63 (s,3H), 3.86 (s,3H), 3.97 (s,3H), 4.03 (s,3H), 7.41 (br s,1H), 7.65 (s,1H) ppm; HRMS 382.1787, calc'd for C$_{23}$H$_{26}$O$_5$ 382.1780; LRMS m/z (rel. intensity) 382 (76), 367 (65), 352 (13), 351 (9), 336 (15), 335 (39).
Preparation of Triacetyl Garveatin A (81):

A solution of garveatin A (77) (6.6mg) in 500 ul pyridine and 500 ul acetic anhydride was stirred overnight at room temperature and evaporated under high vacuum. The reaction mixture was purified by preparative TLC (10% ethyl acetate/CHCl₃) to yield diacetyl garveatin A (82) (1 mg) and triacetyl garveatin A (81) (5.2 mg) as the major reaction products. Compound (82): obtained as a bright yellow oil. R<sub>f</sub> 0.50 in 10% ethyl acetate/hexane; UV (MeOH) 226, 261, 305, 357 nm (sh); IR (CHCl₃) 3050, 2930, 1765, 1708, 1655, 1630 cm<sup>-1</sup>; <sup>1</sup>H NMR (80 MHz,CDCl₃) 1.53 (s,6H), 1.85 (s,3H), 2.35 (s,3H), 2.38 (s,6H), 2.53 (s,3H), 7.24 (s,1H), 7.40 (br s,1H), 10.96 (s,1H) ppm; HRMS 424.1512, calc'd for C<sub>24</sub>H₂₄O₇ 424.1522; LRMS m/z (rel. intensity) 424 (16), 382 (17), 340 (100), 325 (35), 311 (12). Compound (81): obtained as colorless, feathery crystals (from Et<sub>2</sub>0); R<sub>f</sub> 0.3 in 10% ethyl acetate/hexane); UV (MeOH) 226, 264, 305, 317 (sh), 355 (sh) nm; <sup>1</sup>H NMR (80 MHz,CDCl₃) 1.60 (s,6H), 1.80 (s,3H), 2.35 (s,3H), 2.43 (br s,3H), 2.54 (s,3H), 2.55 (s,3H), 7.59 (br s,1H), 7.84 (s,1H) ppm; HRMS 466.1628, calc'd for C<sub>26</sub>H₂₆O₈ 466.1628; LRMS m/z (rel. intensity) 466 (4), 424 (24), 382 (24), 340 (100), 325 (22), 311 (11), 297 (3).

Preparation of Enol Garveatin A triacetate (87):

Triacetyl garveatin A (81) (1 mg) was dissolved in benzene (1 ml) in a screw-cap vial and 2 crystals of p-toluenesulfonic acid were added. The solution was stirred at 60°C for 4 hours and partitioned between 5% K₂CO₃ and ethyl acetate. The ethyl acetate phase was dried with Na₂SO₄ and rotary evaporated to produce
enol garveatin A triacetate (87) in quantitative yield. Compound (87): obtained as long yellow crystals (from 1:1 CHCl$_3$/hexane); $R_f$ 0.52 in 10% ethyl acetate/CHCl$_3$; UV (MeOH) 203, 238, 275 (sh), 403nm; IR (CHCl$_3$) 1763, 1670, 1630, 1605 cm$^{-1}$; $^1$H NMR (400MHz,CDCl$_3$) 1.53 (s,6H), 1.90 (s,3H), 2.05 (s,3H), 2.33 (s,3H), 2.38 (s,3H), 2.55 (br s,3H), 5.00 (d,J=2Hz,1H), 5.38 (d,J=2Hz,1H), 7.24 (s,1H), 7.45 (br s,1H), 15.33 (s,1H) ppm; HRMS 466.1631 calc'd. for C$_{26}$H$_{26}$O$_8$ 466.1628; LRMS m/z (rel. intensity) 466 (6), 424 (21), 382 (37), 340 (100), 325 (56), 311 (12), 296 (14).

Garveatin B (88): Obtained as a yellow oil. $R_f$ 0.50; UV (MeOH) neutral 240 (34200), 260 (16200), 317 (9000), 417 (10600) nm, basic 223 (29800), 243 (40100), 325 (10000), 341 (8900), 417 (14300) nm; IR (CHCl$_3$) 2960, 1710, 1625, 1600, 1430 cm$^{-1}$; $^1$H NMR (CDCL$_3$) 1.18(t, J=7Hz, 3H), 1.62(s, 3H), 2.46(brs, 3H), 2.80(q, J=7Hz, 2H), 7.04(brs, 1H), 7.12(s, 1H), 10.25(s, 1H), 17.15(s, 1H), (diketo tautomer: 1.50(d, J=7Hz, 3H), 3.98(q, J=7Hz, 1H), 9.90(s, 1H), 16.23(s, 1H)); $^1$H NMR (acetone-d$_6$) 1.15(t, J=7Hz, 3H), 1.65(s, 6H), 1.98(s, 3H), 2.43(s,3H), 2.78(q, J=7Hz, 2H), 7.10(s, 1H), 7.31(s, 1H); HRMS 326.1526, calc'd for C$_{20}$H$_{22}$O$_4$ 326.1514; LRMS m/z (rel.intensity) 326 (60), 311 (100), 296 (9), 283 (18), 269 (10), 255 (13).

Preparation of 3-Methyl garveatin B (89):
Garveatin B (88) (5 mg) in diethyl ether was treated with excess diazomethane for 2 hr. Chromatography on silica gel
yielded 3-methyl garveatin B (89) (3 mg). Compound 89: obtained as an orange oil. R_f 0.6 in 80% CHCl_3/hexane; UV (MeOH) 210(12,400), 238(16,100), 265(9,300), 287(8,500), 320sh(3,900), 426(3,800) nm; ^1H NMR (CDCl_3) 1.18(t, J=7Hz, 3H), 1.55(s, 6H), 2.05(s, 3H), 2.44(brs, 3H), 2.80(q, J=7Hz, 2H), 4.00(s, 3H), 7.03(brs, 1H), 7.10(s, 1H), 10.19(s, 1H), 17.08(s, 1H) ppm; LRMS m/z (rel. intensity) 340 (M^+ 56), 325 (100), 309 (24).

Garveatin C (93): Obtained as orange crystals (hexane) m.p. 125°C; R_f 0.54; UV MeOH), 228(13,700), 274(19,700), 305sh(3,300), 389(4,400) nm; IR (CHCl_3) 2930, 1700, 1620, 1455, 1390 cm^{-1}; ^1H NMR (CDCl_3) 1.49(s, 6H), 1.58(s, 6H), 2.38(brs, 3H), 2.62(s, 3H), 3.92(s, 3H), 7.19(s, 1H), 7.35(brs, 1H), 14.45(s, 1H); ^13C NMR (CDCl_3) 211.6(s), 205.8(s), 204.9(s), 163.7(s), 155.9(s), 142.8(s), 140.0(s), 138.0(s), 135.0(s), 125.2(d), 116.3(s), 114.7(d), 108.8(s), 64.1(q), 55.3(s), 48.1(s), 32.4(q), 28.5(q, 2C), 24.6(q, 2C), 19.5(q); HRMS 368.1616, calc'd for C_{22}H_{24}O_{5} 368.1624. LRMS m/z (rel. intensity) 368 (70), 353 (29), 298 (15), 283 (26).

Preparation of Garveatin C acetate (94):

Garveatin C (93) (5mg) was added to 250 ul of acetic anhydride and 250 ul of pyridine and the reaction mixture was stirred overnight at room temperature. The reagents were removed in-vacuo, and the residue was purified via preparative silica TLC(50% ethyl acetate/hexane) to give garveatin C acetate (94) (4 mg). Compound 94: obtained as a pale yellow oil; UV (MeOH) 224, 257, 295, 350 nm; ^1H NMR (CDCl_3) 1.35(s, 6H), 1.55(s, 6H),

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2.37(brs, 3H), 3.84(s, 3H), 7.48(brs, 1H), 7.66(s, 1H); HRMS 410.1735, calc'd for C\textsubscript{24}H\textsubscript{26}O\textsubscript{6} 410.1730; LRMS m/z (rel. intensity) 410 (11), 368 (100), 353 (44), 325 (10), 298 (12).

**Preparation of 9-Methyl garveatin C (95):**

Garveatin A (77) (8 mg) was dissolved in acetone (10 ml) to which K\textsubscript{2}CO\textsubscript{3} and methyl iodide (500 ul) had been added. The reaction mixture was refluxed overnight. The residue obtained after filtration and concentration in-vacuo, was purified by preparative TLC (50% ethyl acetate/hexane) to give 9-methyl garveatin C (95) as the major product (2 mg). Compound 95: obtained as a pale yellow oil. R\textsub{f} 0.50; UV (MeOH) 225, 255, 295sh, 351 nm; \textsuperscript{1}H NMR (CDCl\textsubscript{3}) 1.38(s, 6H), 1.54(s, 6H), 2.38(bs, 3H), 2.61(s, 3H), 3.84(s, 3H), 4.01(s, 3H), 7.45(bs, 1H), 7.52(s, 1H)ppm; LRMS m/z (rel. intensity) 382 (M\textsuperscript{+} 100), 367 (23), 339 (12), 335 (20), 312 (64), 297 (36).

Garveatin C (93) (10 mg) in 10 ml of HPLC grade acetone was treated with K\textsubscript{2}CO\textsubscript{3} (anhydrous, 130mg) and methyl iodide (500ul). The reaction mixture was refluxed for 7 h. The reaction mixture was filtered and evaporated to give a single product which was identical by TLC, UV, \textsuperscript{1}H NMR, and mass spectral comparison to the 9-methyl garveatin C sample prepared from garveatin A as described above.

**Garveatin D (96):** obtained as a yellow solid. R\textsub{f} 0.40; UV(MeOH) 238, 269, 417nm; \textsuperscript{1}H NMR(300 MHz,CDCl\textsubscript{3}) 1.50(s,6H), 1.58(s,6H), 2.41(br s,3H), 2.63(s,3H), 7.05(br s,1H), 7.12(s,1H),
10.20(s,1H), 16.15(s,1H)ppm; HRMS 354.1465, calc'd for C_{21}H_{22}O_{5} 354.1468; LRMS m/z (rel. intensity) 354(M^+,53), 339(59), 321(30), 311(15), 297(13), 293(16).

Preparation of 2-Hydroxygarveatin A triacetate (98):

Fraction D (200mg) obtained from flash chromatography of the methylene chloride phase of the extract was dissolved in pyridine(3ml) and acetic anhydride(7ml) and stirred at room temperature for 48 hours. After evaporation of the reagents, the residue was purified by flash chromatography (methanol/methylene chloride gradient) and preparative TLC (50% ethyl acetate/hexane) to give 2-hydroxygarveatin A triacetate (98) (90mg). Triacetate (98): obtained as a colourless solid. R_f 0.25; UV (MeOH) 226, 260, 303, 355nm; \textsuperscript{1}H NMR (400 MHz,CDCl\textsubscript{3}) 1.45(s,3H), 1.57(s,3H), 1.79(s,3H), 2.20(s,3H), 2.34(s,3H), 2.41(br s,3H), 2.50(s,3H), 7.61(s,1H), 7.79(s,1H)ppm; HRMS 482.1567, calc'd. for C_{26}H_{26}O_{9} 482.1577; LRMS m/z (rel. intensity) 482(M^+,3), 440(25), 398(100), 356(20), 338(32), 314(83), 313(77).

Preparation of diacetate (99):

On standing in pyridine-d\textsubscript{6} in an NMR tube overnight, triacetate 98 partially decomposed to diacetate 99 (yield 40%) which was purified in via flash chromatography (ethyl acetate/hexane gradient). Diacetate 99: R_f 0.40; UV (MeOH) 226, 272, 306(sh), 385nm; \textsuperscript{1}H NMR(80 MHz,CDCl\textsubscript{3}) 1.58(s,3H), 1.63(s,3H), 1.74(s,3H), 2.24(s,3H), 2.40(s,3H), 2.45(br s,3H), 2.55(s,3H), 7.26(1H), 7.47(s,1H), 9.26(s,1H)ppm; \textsuperscript{13}C NMR(75 MHz,CDCl\textsubscript{3}) 206.5, 202.7, 198.4, 169.7, 163.7, 145.0, 142.8,
139.6, 137.9, 134.1, 126.9, 116.3, 115.1, 108.1, 82.6, 47.4, 32.0, 31.2, 29.3, 23.4, 21.1, 20.0, 19.7 ppm; MS m/z (rel. intensity) 440 (M⁺, 14), 398 (71), 356 (9), 340 (14), 338 (25), 314 (90), 313 (100), 295 (31).

Preparation of 8,9-Dimethyl Garveatin A (100):

Trimethyl garveatin A (79) (10 mg) was dissolved in 5 mls 50% aq. acetic acid in a round-bottomed flask and refluxed for 10 minutes. The reaction mixture was diluted with H₂O and partitioned with CH₂Cl₂. The CH₂Cl₂ phase was dried with Na₂SO₄ and rotary-evaporated to give a quantitative yield of 8,9-dimethyl garveatin A (100). Compound 100: obtained as a light yellow oil. Rᵋ 0.54; ¹H NMR (300 MHz, CDCl₃) 1.54 (s, 6H), 2.01 (s, 3H), 2.39 (br s, 3H), 2.61 (s, 3H), 3.83 (s, 3H), 4.03 (s, 3H), 7.45 (br s, 1H), 7.63 (s, 1H) ppm; MS m/z (rel. intensity) 368 (M⁺ 100), 353 (51), 342 (10), 341 (11), 325 (80).

Preparation of 2-Acetoxy,8,9-dimethyl Garveatin A (101):

8,9-dimethyl garveatin A (100) (10 mg) was stirred in glacial acetic acid (10 ml) containing excess Pb(OAc)₄ (60 mg) at room temperature. After 16 hours the reaction mixture was diluted with H₂O and partitioned with CH₂Cl₂. The organic phase was dried with Na₂SO₄ and purified by preparative TLC (5% ethyl acetate/hexane) to yield 2-acetoxy,8,9-methyl garveatin A (101) (6 mg) as the major reaction product. Compound 101: obtained as a colorless oil. Rᵋ 0.44; UV (MeOH) 261, 300 (sh), 356 nm; ¹H NMR (80 MHz, CDCl₃) 1.47 (s, 3H), 1.57 (s, 3H), 1.76 (s, 3H), 2.23
(s,3H), 2.37 (br s,3H), 2.58 (s,3H), 3.80 (s,3H), 4.00 (s,3H), 7.43 (br s,1H), 7.57 (s,1H) ppm; MS m/z (rel. intensity) 426 (M+48), 398 (5), 384 (42), 368 (14), 353 (9), 342 (100), 341 (92), 327 (56).

2-Hydroxygarveatin B (102): obtained as a yellow solid. Rf 0.28; UV (MeOH) 224, 275, 423nm; 1H NMR (80 MHz,CDCl3) 1.18(t,3H, J=7Hz), 1.50(s,3H), 1.65(s,3H), 1.78(s,3H), 2.47(br s,3H), 2.80(q,2H, J=7Hz), 4.60(bs, 1H0, 7.08(br s,1H), 7.13(s,1H), 9.68(s,1H), 15.15(s,1H)ppm; HRMS 342,1467, calcd. for C20H22O5 342.1468; LRMS m/z (rel. intensity) 342(M+15), 327(5), 300(26), 299(30), 285(13).

Preparation of 2-Hydroxygarveatin B acetates:

A solution of 2-Hydroxygarveatin B (102) (16mg), pyridine(0.25ml), and acetic anhydride(0.5ml) was stirred overnight at room temperature. After removal of the reagents in-vacuo, the residue was chromatographed on silica (2% methanol/methylene chloride) to give 2-hydroxygarveatin B diacetate (103) (3mg), and 2-hydroxygarveatin B triacetate (104) (11mg). Diacetate 103: obtained as a light yellow oil. Rf 0.30; UV (MeOH) 213, 250, 307(sh), 319, 363nm; 1H NMR (80 MHz,CDCl3) 1.15(t,3H, J=7Hz), 1.26(s,3H),1.40(s,3H), 1.83(s,3H), 2.45(s,3H), 2.47(s,3H), 2.52(br s,1H), 2.71(q,1H J=7 Hz), 4.63(br s,1H), 7.62(br s,1H), 7.66(s,1H)ppm; MS m/z (rel. intensity) 426(M+10), 384(19), 342(47), 326(18), 324(33), 300(84), 299(100), 282(77).

Triacetate (104): obtained as a colorless oil. Rf 0.40; UV (MeOH)
217, 260, 304, 316, 359nm; $^1$H NMR(80 MHz, CDCl$_3$)
1.13(t, 3H, J=7Hz), 1.44(s, 3H), 1.55(s, 3H), 1.78(s, 3H), 2.22(s, 3H), 2.45(s, 6H), 2.50(br s, 3H), 2.70(q, 2H, J=7Hz), 7.55(br s, 1H), 7.70(s, 1H) ppm; HRMS 468.1776, calcd. for C$_{26}$H$_{28}$O$_6$ 468.1784; LRMS m/z (rel. intensity) 468(M$^+$, 4), 426(20), 384(100), 342(19), 324(28), 300(44), 299(82), 282(26).

Garveatin A quinone (105): obtained as a red oil. R$_f$ 0.49; UV(MeOH) 218, 265, 280(sh) and 410nm; $^1$H NMR(CDCl$_3$, 80MHz) 1.63(s, 6H), 1.98(s, 3H), 2.41(bs, 3H), 2.60(s, 3H), 7.55(bs, 1H), 11.45(s, 1H), 11.66(s, 1H); $^{13}$C NMR (CDCl$_3$, 75MHz) 202.1, 200.0, 193.0, 180.4, 159.5, 159.1, 156.2, 147.2, 136.3, 131.9, 127.7, 123.0, 118.6, 112.4, 48.3, 31.9, 26.4(2 carbons), 20.5, 8.1; HRMS 354.1108, calc'd for C$_{20}$H$_{18}$O$_6$ 354.1103; LRMS m/z (rel. intensity) 354 (M$^+$ 95), 339 (13), 326 (31), 311 (44), 297 (63), 283 (66), 269 (23).

Garveatin B quinone (106): Obtained as a red oil; R$_f$ 0.63; UV (MeOH) 208, 231, 283, 416nm; $^1$H NMR (400 MHZ, CDCl$_3$) 1.16(t, J=7Hz, 3H), 1.61(s, 3H), 1.97(s, 3H), 2.45(s, 3H), 2.78(q, J=7Hz, 2H), 7.51(s, 1H), 11.79(s, 1H), 11.84(s, 1H) ppm; HRMS 340.1306, calc'd for C$_{20}$H$_{20}$O$_5$: 340.1311; LRMS m/z (rel. intensity) 340(M+, 100), 326(20), 312(44), 297(38), 284(51), 269(55), 266(42).

Preparation of garveatin B dimer (108):

A solution of garveatin B (88) in CDCl$_3$ was allowed to stand overnight in a closed NMR tube at room temperature. The sample
was then chromatographed on preparative TLC (20% ethyl acetate/hexane) to give garveatin B dimer \((\text{108})\) in approx. 30% yield. \textbf{Dimer 108}: obtained as an orange oil; \(R_f\) 0.66; UV (MeOH) 225, 275, 320 sh, 434 nm; \(^1\)H NMR (270 MHz, CDCl\(_3\)) 1.07 (t, \(J=7\)Hz, 3H), 1.38 (s, 3H), 1.66(s,3H), 1.88 (s,3H), 2.43 (s,3H), 2.53 (m,1H), 2.70 (m,1H), 6.98( s,2H), 9.37 (s,1H), 15.79 (s,1H) ppm; \(^{13}\)C NMR (75 MHz, C\(_6\)D\(_6\)) 208.9, 202.4, 167.3, 155.6, 143.5, 140.0, 137.7, 126.4, 119.9, 115.7, 110.9, 107.9, 63.0, 48.6, 33.6, 25.9, 21.2, 20.4, 19.8, 13.8 ppm; MS m/z (rel.intensity) 326 (57), 311 (100), 283 (20), 269 (11) (molecular ion not seen in EI spectrum).

\textbf{Preparation of garveatin B dimer tetraacetate (109)}:

Garveatin B dimer \((\text{108})\) (2 mg) was treated with 100 ul of acetic anhydride and 100 ul of pyridine overnight. The reaction mixture was then chromatographed on preparative TLC (50% ethyl acetate/hexane) to give the tetraacetate \textbf{109} in quantitative yield. \textbf{Tetraacetate 109}: obtained as a light yellow oil; \(R_f\) 0.60; \(^1\)H NMR (300 MHz, CDCl\(_3\)) 1.15 (t, \(J=7\)Hz,3H), 1.56 (s,3H), 1.71 (s,3H), 1.85 (s,3H), 2.30 (s,3H), 2.42 (s,3H), 2.52 (s,3H), 2.55 (m,1H), 2.75 (m,1H), 7.61 (s,1H), 7.75 (s,1H) ppm; MS m/z (rel. intensity) 818 (M\(^+\),0.02), 776 (0.30), 734 (0.31), 716 (0.44), 674 (2), 410 (3), 368 (12), 326 (100), 311 (67).

\textbf{Preparation of garveatin A dimer (110)}:

Garveatin A \((\text{77})\) (10 mg) was dissolved in CH\(_3\)CN (25 ml) in a screw-cap vial and 0.5 ml of conc. HCl was added. The mixture was
stirred overnight under a stream of air at room temp. Preparative TLC of the reaction mixture (5% MeOH/CHCl$_3$) gave garveatin A dimer (110) (6 mg) as the major product. **Dimer (110):**

- obtained as a light yellow oil; $R_f$ 0.38; UV (MeOH) 231, 270, 422 nm (neutral), 245 sh, 270, 428 nm (basic); $^1$H NMR (80 MHz, CDCl$_3$) 1.47 (s,3H), 1.59 (s,3H), 1.94 (s,3H), 2.38 (br s,3H), 2.58 (s,3H), 6.98 (br s,1H), 7.05 (s,1H), 10.34 (s,1H), 15.81 (s,1H) ppm; MS m/z (rel. intensity) 340 (51), 325 (100), 310 (12), 297 (13) (no molecular ion was detected in the EI spectrum).

**Preparation of garveatin A dimer tetraacetate (111):**

Garveatin A dimer (110) (3 mg) was treated with 500 ul of acetic anhydride and 500 ul of pyridine and stirred overnight at room temperature. The tetraacetate 111 (1 mg) was isolated as the major reaction product. **Tetraacetate 111:** obtained as a colorless oil; $R_f$ 0.15; $^1$H NMR (300 MHz, CDCl$_3$) 1.72 (s,3H), 1.85 (s,3H), 2.32 (s,3H), 2.35 (s,3H), 2.44(s,3H), 2.51 (s,3H), 7.64 (br s,1H), 7.79 (s,1H) ppm; MS m/z (rel. intensity) 846 (M$^+$ 0.14), 804 (0.56), 762 (0.60), 720 (0.42), 424 (4), 382 (16), 340 (94), 325 (74).

**Preparation of Desacetyl Garveatin A (116):**

Garveatin A (77) (20 mg) was refluxed in a solution of 1:1 glacial acetic acid/conc. HCl (10 ml) for 10 minutes. The reaction mixture was diluted with H$_2$O and extracted with CH$_2$Cl$_2$. The organic phase was washed with 5% aq. bicarbonate, concentrated and purified by preparative TLC (10% ethyl acetate/CHCl$_3$) to yield desacetyl garveatin A (116) (10 mg) and
unreacted garveatin A (9 mg). Compound (116): obtained as a yellow oil. R_f 0.50; UV (MeOH) neutral/basic 239, 320, 343 (sh), 410, 430 nm (sh), acidic 234, 256, 294 (sh), 319 (sh), 385 nm (sh); ^1H NMR (300 MHz,CDCl_3) keto-enol tautomer: 1.63 (s,6H), 1.98 (s,3H), 2.43 (s,3H), 6.68 (br d,J(meta)=1.6Hz,1H), 7.00(d,J(meta)=1.6Hz,1H), 7.16 (s,1H), 9.98 (s,1H), 17.00 (s,1H), diketo tautomer: 1.50 (d,J=7Hz,3H), 1.61 (s,3H), 1.62 (s,3H), 2.44 (s,3H), 4.00 (q,J=7Hz,1H), 6.75 (d,J=1.6Hz,1H), 7.01 (d,J=1.6Hz,1H), 7.15 (s,1H), 9.62 (s,1H), 16.00 (s,1H) ppm; MS m/z (rel.intensity) 298 (M^+ 100), 283 (94), 255 (69), 242 (29), 214 (34).

Preparation of Desacetyl Garveatin A Quinone (117):
Garveatin A quinone (105) (10 mg) was refluxed in 10 mls of 1:1 glacial acetic acid/conc.HCl for 10 minutes and worked up as for the previous reaction. The reaction mixture was purified by preparative TLC (10% ethyl acetate/CHCl_3) to yield desacetyl garveatin A quinone (117) (6 mg). Compound (117): obtained as a red oil. R_f 0.55; UV (MeOH) neutral 210, 233, 255 (sh), 282 (sh), 422 nm, basic 270, 307, 490 nm; ^1H NMR (400 MHz,CDCl_3) 1.62 (s,6H), 1.98 (s,3H), 2.48 (br s,3H), 7.11 (br s,J(meta)=1.6Hz,1H), 7.52 (br s,J(meta)=1.6Hz,1H), 11.38 (s,1H), 11.71 (s,1H) ppm; MS m/z (rel. intensity) 312 (M^+ 78), 297 (6), 284 (44), 269 (92), 256 (100), 241 (61), 228 (30), 227 (30), 213 (35).

2-hydroxygarvin A (119): Obtained as pale yellow needles
(diethyl ether) mp 195°C; R_f 0.33; UV (MeOH) neutral 226, 278, 318, 332, 385 nm, basic 226, 278, 332, 407 nm; IR (CHCl_3) 3490, 2990, 1720, 1700 sh, 1610, 1380, 1210 cm^{-1}; \(^1\)H NMR (CDCl_3) 1.04(t, J=7Hz, 3H), 1.52(s, 3H), 1.66(s, 3H), 1.73(s, m, 2H), 1.81(s, 3H), 3.15(m, 2H), 3.98(s, 3H), 3.99(s, 3H), 6.98(s, 1H), 7.21(s, 3H), 14.25(s, 1H); \(^1^3\)C NMR (CDCl_3) 209.9(s), 199.9(s), 168.3(s), 166.0(s), 157.7(s), 142.9(s), 142.1(s), 141.9(s), 126.9(s), 116.7(s), 116.5(d), 107.3(s), 105.0(d), 81.6(s), 55.9(q), 52.3(q), 46.9(s), 36.0(t), 30.6(q), 28.9(q), 28.2(q), 25.3(t), 14.7(q); HRMS observed 414.1677, calc'd for C_{23}H_{26}O_7 414.1679; LRMS m/z (rel. intensity) 414 (28), 398 (12), 383 (14), 372 (79), 371 (100), 357 (39), 339 (29).

**Preparation of 2-hydroxygarvin A diacetate (120):**

2-hydroxygarvin A (119) (5 mg) was dissolved in 500 ul of acetic anhydride and 500 ul of pyridine and stirred overnight at room temperature. The reagents were removed in-vacuo and the residue was purified via silica gel prep TLC (10% ethyl acetate/chloroform) to yield 2-hydroxygarvin A diacetate (120) (4 mg). Compound 120: obtained as a pale yellow oil; R_f 0.35; UV (MeOH) 226, 268, 327, 356(sh) nm; \(^1\)H NMR (CDCl_3) 1.03(t, J=7Hz, 3H), 1.50(s, 3H), 1.60(s, 3H), 1.70(m, 2H), 1.83(s, 3H), 2.25(s, 3H), 2.94(m, 2H), 3.99(s, 6H), 7.06(s, 1H) 7.73(s, 1H); HRMS 498.1889, calc'd for C_{27}H_{30}O_9 498.1890; LRMS m/z (rel. intensity) 498 (M^+ 6), 456 (48), 396 (32), 372 (8), 371 (10), 344 (12).

**Garvin A quinone (122):** Obtained as a red oil; R_f 0.50; UV (MeOH) 216, 276, 384 nm; \(^1\)H NMR (CDCl_3) 1.05(t, J=7Hz, 3H), 1.60(m, 2H),
1.61(s, 6H), 1.98(s, 3H), 2.95(m, 2H), 3.98(s, 3H), 4.03(s, 3H), 7.56(s, 1H), 12.15(s, 1H); HRMS observed M⁺ m/z 412.1518, C_{23}H_{24}O_{7} requires 412.1522. LRMS m/z (rel. intensity) 412 (100), 398 (6), 397 (6), 384 (41), 369 (15), 341 (21).

**Garvin A quinone acetate (123):**

Garvin A quinone (122) was dissolved in acetic anhydride (250 ul) and pyridine (250 ul) and stirred overnight at room temperature. The reagents were removed in-vacuo and the residue was purified by preparatory TLC (50% ethyl acetate/hexane) to yield garvin A quinone acetate (123) (3 mg). Compound 123: obtained as light yellow needles (acetone); R_f 0.55; UV (MeOH) 224, 276, 299sh, 344sh nm; ¹H NMR (CDCl₃) 1.02(t, J=7Hz, 3H), 1.59(m, 2H), 1.61(s, 6H), 1.94(s, 3H), 2.37(s, 3H), 2.83(bs, 2H), 3.94(s, 3H)< 3.96(s, 3H), 7.48(s, 1H); HRMS 454.1632, calc'd for C_{25}H_{26}O_{8} 454.1628; LRMS m/z (rel. intensity) 454 (35), 423 (6), 412 (64), 397 (20), 384 (31), 341 (12).

**Garvin A (124):** obtained as a yellow solid. R_f 0.41; UV (MeOH) 220, 247, 274, 321, 383 nm; ¹H NMR(300 MHz,CDCl₃) 1.03(t,3H,J=7Hz), 1.64(s,3H), 1.73(m,2H), 1.99(s,3H), 3.18(m,2H), 3.95(s,3H), 3.97(s,3H), 6.91(s,1H), 7.17(s,1H), 15.14(s,1H), also 1.50(d,3H,J=7Hz), 4.01(q,1H,J=7Hz) due to diketo tautomer; HRMS 398.1724, calc'd for C_{23}H_{26}O_{6} 398.1730; LRMS m/z (rel. intensity) 398(M⁺,100), 383(30), 367(21), 351(17).
Preparation of Monomethyl Garvin A (125):

Garvin A (124) (14mg) was dissolved in diethyl ether (3 ml, anhyd.) and treated with diazomethane (generated by adding 0.6 ml 5M NaOH to 100mg N-methylnitro-nitroso-guanidine). After standing overnight, the reaction mixture was purified by preparative TLC (80% CHCl₃/hexane) to give monomethylgarvin A (125) (6.4mg) as the major product. **Compound (125):** R_{f} 0.60; UV (MeOH) 220, 250, 275, 293(sh), 322, 334(sh), 390nm. \(^{1}H\) NMR (300 MHz,CDCl₃) 1.02(t,3H), 1.55(s,6H), 1.73(m,2H), 2.06(s,3H), 3.19(m,2H), 3.93(s,3H), 3.96(s,3H), 3.99(s,3H), 6.90(s,1H), 7.14(s,1H), 15.87(s,1H)ppm; MS m/z (rel. intensity) 412(M^+,44), 397(32), 381(15), 365(24), 337(10).

Garvin B (126): obtained as a yellow solid. R_{f} 0.43; UV (MeOH) 229, 256, 289, 327, 389nm; \(^{1}H\) NMR(300 MHz, DMSO-d₆) 1.50(d,3H,J=7Hz), 1.54(s,3H), 1.58(s,3H), 1.85(s,3H), 3.23(dd,1H,J=19,13Hz), 4.35(dd,1H, J=19,3Hz), 4.77(m,1H), 7.16(s,1H), 7.35(s,1H), 11.14(s,1H), 17.85(s,1H)ppm; \(^{13}C\) NMR(75 MHz, DMSO d₆) 191.05, 177.97, 170.58, 166.33, 159.05, 149.09, 145.56, 142.55, 115.00, 114.40, 110.53, 110.50, 107.70, 107.65, 75.40, 48.24, 33.97, 29.24, 28.93, 20.43, 7.60ppm; HRMS 368.1269, calc'd. for C_{21}H_{20}O_{6} 368.1260; LRMS m/z (rel. intensity) 368(M^+,6), 353(3), 325(3), 311(3).

Preparation of Trimethyl Garvin B (129):

A solution of garvin B (126) (15mg), K₂CO₃(100mg), and methyl iodide(0.5ml) in 10ml acetone was was refluxed under a stream of N₂ for 6 hrs and then partitioned between water and
methylene chloride. The methylene chloride soluble material was chromatographed on silica (50% ethyl acetate/hexane) to yield trimethylgarvin B (129) (10mg) as the major reaction product.

**Compound (129):** obtained as a pale yellow solid. $R_f 0.27$; $[\alpha]_D^{22} +172.69^\circ$ (c0.26, CHCl$_3$); UV (MeOH) 232, 275, 330nm; $^1$H NMR (300 MHz, CDCl$_3$) 1.37(s,3H), 1.42(s,3H), 1.54(s,3H), 1.55(d,3H, $J=7$Hz), 1.57(s,3H), 3.27(dd,1H, $J=19,13$Hz), 3.89(s,3H), 4.04(s,3H), 4.13(dd,1H, $J=19,3$Hz), 4.45(m,1H), 7.13(s,1H), 7.50(s,1H)ppm; MS m/z (rel. intensity) 410(M$^+$,100), 395(11), 377(13), 367(12), 340(49), 339(40).

**Preparation of Garvin B diacetate (127) and triacetate (128):**

Garvin B (126) (30mg) was acetylated at room temperature with pyridine (0.5ml) and acetic anhydride (0.5ml). After evaporation of the reagents, the residue was purified by preparative TLC (10% ethyl acetate/methylene chloride) and HPLC (10% ethyl acetate/hexane) to give garvin B diacetate (127) (5mg) and garvin B triacetate (128) (15mg). Compound 127: obtained as a light yellow oil. $R_f 0.38$; UV (MeOH) 245, 286, 389nm. $^1$H NMR (80 MHz, CDCl$_3$) 1.55(s,3H), 1.57(s,3H), 1.58(d,3H, $J=7$Hz), 1.88(s,3H), 2.38(s,3H), 2.41(s,3H), 3.40(dd,1H, $J=19,13$Hz), 4.32(dd,1H, $J=19,3$Hz), 4.60(m,1H), 7.25(s,1H), 7.30(s,1H), 15.75(s,1H)ppm; MS m/z (rel. intensity) 452(M$^+$,2), 451(5), 409(15), 367(76), 352(23). Compound 128: obtained as a pale yellow oil. $R_f 0.24$; UV (MeOH) 222, 237, 281, 292, 305(sh), 345, 365(sh), 384nm; $^1$H NMR (400 MHz, CDCl$_3$) 1.57(d,3H, $J=7$Hz), 1.58(s,3H), 1.64(s,3H), 1.81(s,3H), 2.36(s,3H), 2.40(s,3H), 2.40(s,3H),
2.57(s,3H), 3.28(dd,1H,J=19,13Hz), 3.85(dd,1H,J=19,3Hz),
4.58(m,1H), 7.48(s,1H), 7.86(s,1H)ppm; MS m/z (rel. intensity)
494(M^+,1), 493(1), 451(13), 409(22), 367(68), 352(16).

2-Hydroxygarvin B (130): obtained as a yellow solid. R_f 0.35; UV
(MeOH) 234, 287, 386 nm (neutral), 253, 310, 417 nm (basic); ^1H
NMR(400 MHz, CDCl_3) 1.50, 1.54(s,3H), 1.64, 1.65(d, J=7Hz,3H), 1.65,
1.66(s,3H), 1.80, 1.82(s,3H), 3.41, 3.44(dd, J=12,19Hz,1H),
4.02(br s,1H), 4.38(dd, J=3,19Hz,1H), 4.74(m,1H), 7.11(s,2H),
11.44, 11.45(s,1H), 14.61, 14.62(s,1H)ppm; ^13C NMR(75 MHz, CDCl_3)
209.83, 199.71(199.55), 169.85(169.80), 167.39(167.17),
161.27(167.16), 145.40(145.26), 144.58(144.54), 143.84, 116.03,
115.94, 111.81, 110.81(110.77), 107.00(106.88), 81.64(81.57),
75.43(75.35), 47.14(47.05), 34.60(34.36), 30.70(30.44),
29.15(29.04), 28.43(27.99), 20.83 ppm; HRMS 384.1214, calc'd for
C_{21}H_{20}O_7 384.1209; LRMS m/z (rel. intensity) 384(M^+,32),
368(22), 353(9), 342(90), 341(100), 327(61), 309(26).

Preparation of Triacetates (131a,b) of 2-hydroxygarvin B:

2-hydroxygarvin B (130) (10 mg) was treated with 300ul of
acetic anhydride and 300ul of pyridine and stirred at room
temperature for 16 hours. After evaporation of the solvents, the
reaction mixture was purified by repeated preparative TLC (x3) in
50% ethyl acetate/hexane to yield the diastereomeric triacetates
131a (4 mg) and 131b (4 mg). Triacetate 131a: obtained as a
colorless oil. R_f 0.42; UV (MeOH) 230, 269, 358 nm; ^1H NMR (270
MHz, CDCl_3) 1.47(s,3H), 1.58(s,3H), 1.58(d,J=7Hz,3H), 1.84
(s,3H), 2.22(s,3H), 2.39(s,3H), 2.45(s,3H), 3.20(m,1H), 3.92
(m,1H), 7.50 (s,1H), 7.80 (s,1H) ppm; MS m/z (rel. intensity) 510 (M^+ 2), 468 (19), 426 (100), 384 (10), 366 (40), 342 (28), 341 (42). Triacetate 131b: obtained as a colorless oil. R_f 0.39; UV (MeOH) 230, 268, 357 nm; ^1H NMR (270 MHz,CDCl_3) 1.49 (s,3H), 1.55 (d,J=7Hz,3H), 1.63 (s,3H), 2.24 (s,3H), 2.41 (s,3H), 2.50 (s,3H), 3.27 (m,1H), 3.68 (m,1H), 4.58 (m,1H), 7.50 (s,1H), 7.80 (s,1H) ppm; MS m/z (rel. intensity) 510 (M^+ 1), 468 (18), 426 (100), 384 (10), 366 (43), 342 (31), 341 (43).

Garvin B quinone (132): obtained as a red oil. R_f 0.48; UV (MeOH) neutral 225 (41400), 292 (30800), 397 (7300) nm, basic 237 (37700), 270 (30000), 310 (25000), 354 (sh) (9900), 463 (8000) nm; ^1H NMR (80 MHz,CDCl_3) 1.58 (s,3H), 1.60 (s,3H), 1.63 (d,J=7Hz,3H), 1.95 (s,3H), 3.23 (dd,J=19,12 Hz,1H), 3.99 (dd,J=19,3 Hz,1H), 4.72 (m,1H), 7.64 (s,1H), 11.85 (s,1H), 12.28 (s,1H) ppm; ^13C NMR (75 MHz,CDCl_3) 200.04, 189.51, 180.43, 169.07, 167.25, 159.92, 153.71, 145.65, 139.11, 128.72, 118.96, 118.73, 116.22, 112.58, 75.44, 47.70, 33.35, 26.54, 25.57, 20.72, 8.11 ppm; HRMS 382.1055, calc'd for C_{21}H_{18}O_{7} 482.1052; LRMS m/z (rel. intensity) 382 (M^+ 100), 354 (47), 339 (29), 326 (10), 325 (6), 311 (18), 308 (22).

Preparation of Garvin B Quinone diacetate (133):

Garvin B quinone (132) (7 mg) was reacted with 500ul of acetic anhydride and 500ul of pyridine and stirred overnight at room temperature. Evaporation of the solvents under high vacuum gave the diacetate 133 in quantitative yield. Diacetate 133:
obtained as a pale yellow oil. \( R_f \) 0.47; UV (MeOH) neutral 215, 245 (sh), 296 (sh), 335 nm, basic 228, 249, 288, 426 nm; \(^1\)H NMR (300 MHz, CDCl\(_3\)) 1.56 (d, \( J=7 \) Hz, 3H), 1.59 (s, 3H), 1.68 (s, 3H), 1.99 (s, 3H), 2.38 (s, 6H), 3.12 (dd, \( J=18,11 \) Hz, 1H), 3.50 (m, 1H), 4.60 (m, 1H), 7.75 (s, 1H) ppm; MS m/z (rel. intensity) 466 (M+ 0.1), 424 (23), 382 (37), 354 (27), 339 (15), 308 (10).

**Preparation of garvin A dimer 134:**

Garvin A \((\text{124})\) (4 mg) was dissolved in MeOH (10 ml) and 50 mg Ag\(_2\)O was added. The mixture was stirred under a stream of air for 24 hours and purified by preparative TLC (50% ethyl acetate/hexane) to give garvin A dimer \((\text{134})\) (2 mg) as the major product. Dimer 134: obtained as a yellow oil; \( R_f \) 0.43; UV (MeOH) 228, 277, 332, 393 nm; \(^1\)H NMR (300 MHz, CDCl\(_3\)) 0.93 (t, \( J=7\) Hz, 3H), 1.58 (s, 3H), 1.60 (m, 2H), 1.71 (s, 3H), 1.94 (s, 3H), 2.90 (m, 2H), 3.94 (s, 6H), 6.89 (s, 1H), 7.11 (s, 1H), 14.65 (s, 1H) ppm; \(^{13}\)C NMR (75 MHz, CDCl\(_3\)) 209.9, 202.5, 168.5, 166.9, 157.2, 142.9, 142.6, 141.7, 125.2, 116.4, 115.2, 108.0, 104.7, 63.4, 55.8, 52.3, 47.9, 35.9, 32.1, 28.2, 24.9, 21.7, 14.8 ppm; MS m/z (rel. intensity) 398 (100), 383 (51), 367 (36), 351 (34) (molecular ion not seen in EI spectrum).

**Hydrogenolysis of garvin A dimer:**

Garvin A dimer \((\text{134})\) (4 mg) was dissolved in 95% EtOH (20ml) in an erlenmeyer flask. A spatula of palladium on activated charcoal was added, and the flask was sealed under an atmosphere of hydrogen gas and stirred overnight. A quantitative yield of garvin A \((\text{124})\) was obtained.
Preparation of garvin A dimer diacetate 135:

Treatment of garvin A dimer (134) (5 mg) with 250 ul of acetic anhydride and 250 ul of pyridine overnight at room temp. yielded the diacetate 135 (3 mg) as the major reaction product. Diacetate 135: obtained as a colorless oil; $R_f$ 0.61; UV (MeOH) 211, 268, 324, 355 (sh) nm; $^1H$ NMR (300 MHz,CDCl$_3$) 1.02 (t, J=7Hz,3H), 1.55 (s,3H), 1.60 (m,2H), 1.70 (s,3H), 1.86 (s,3H), 2.32 (s,3H), 2.91 (m,2H), 3.95 (s,3H), 3.96 (s,3H), 7.08 (s,1H0, 7.72 (s,1H) ppm; MS m/z (rel. intensity) 878 (M$^+$,0.24), 836 (2), 794 (2), 398 (100), 383 (17), 369 (32).

Garveatin B-garvin B dimer 136: Obtained as an orange oil; $R_f$ 0.65; UV (MeOH) 283, 400 nm (neutral), 282, 305(sh) 414 nm (basic); $^1H$ NMR (400 MHz,CDCl$_3$) 1.00 (t, J=7Hz,3H), 1.20 (s,3H), 1.24 (s,3H), 1.51 (d, J=7Hz,3H), 1.78 (s,3H), 1.80 (s,3H), 1.81 (s,3H), 1.82 (s,3H), 2.35 (dd, J=19,12Hz,1H), 2.42 (s,3H), 2.42 (m,1H), 2.77 (m,1H), 3.83 (dd, J=19,3Hz,1H), 4.40 (m,1H), 6.90 (s,2H), 6.92 (s,1H), 7.02 (s,1H), 9.10 (s,1H), 14.85 (s,1H), 15.70 (s,1H) ppm; MS m/z rel. intensity) 368 (100), 353 (53), 326 (53), 311 (88), 297 (20) (no molecular ion was seen in the EI spectrum); FAB MS (MeOH solution in thioglycerol) m/z 693(M+1 ion), 649, 369, 368, 327, 326, 311, 297.

Garvalone A (137): obtained as a light yellow oil. $R_f$0.42; UV (MeOH) 228(15,000), 279(20,100), 317(4,900), 330(4,100), 382(4,400) nm; IR (CHCl$_3$) 1570, 1610, 1718, 1737 cm$^{-1}$; $^1H$ NMR(300
MHz, CDCl$_3$)  1.11(t, 3H, J=7Hz),  1.47(s, 3H),  1.55(s, 3H),
1.59(s, 3H),  1.75(m, 2H),  2.04(s, 3H),  2.23(m, 4H),  3.15(m, 2H),
3.93(s, 3H),  3.96(s, 3H),  6.93(s, 1H),  7.13(s, 1H),  15.10(s, 1H)ppm;
$^{13}$C NMR (75 MHz, CDCl$_3$)  211.90(s),  207.04(s),  203.88(s),
168.61(s),  166.59(s),  157.35(s),  142.97(s),  142.50(s),  141.82(s),
126.45(s),  116.78(s),  115.28(d),  108 70(s),  104.96(d),  58.30(s),
55.94(q),  52.49(q),  47.85(s),  39.06(t),  36.14(t),  31.43(t),
30.52(q),  29.86(q),  27.01(q),  25.42(t),  24.21(q),  14.80(q)ppm;
HRMS 468.2162, calc'd for C$_{27}$H$_{32}$O$_7$ 468.2149; LRMS m/z (rel. intensity) 468(M$^+$, 94), 453(14), 437(14), 398(100), 397(89),
383(43).

**Preparation of Methyl Garvalone A (139):**

Garvalone A (137) (20mg), $\text{K}_2\text{CO}_3$ (150 mg, anhyd.), and methyl iodide (0.6ml) were dissolved in 10ml of acetone and the mixture was stirred at reflux overnight under an atmosphere of N$_2$. The reaction mixture was rotary-evaporated and partitioned between water and methylene chloride. The methylene chloride phase yielded methyl garvalone A (139) (20mg). Compound (139) obtained as a pale yellow oil. $R_f$ 0.39; UV (MeOH) 225, 263, 314nm; $^1$H NMR(400 MHz, CDCl$_3$)  0.94(t, 3H, J=7Hz),  1.36(s, 3H),
1.52(s, 3H),  1.57(s, 3H),  1.6(m, 2H),  1.95-2.10(m, 2H),  2.05(s, 3H),
2.24(m, 2H), 2.48(m, 2H),  2.87(m, 1H),  3.14(m, 1H),  3.95(s, 3H),
3.97(s, 3H),  7.02(s, 1H),  7.49(s, 1H)ppm; MS m/z (rel. intensity) 482(M$^+$, 57), 467(11), 451(9), 426(23), 412(38), 411(37), 397(62),
383(18).
Preparation of 2-(3-oxobutyl) garveatin A (140):

To a solution of garveatin A (77) (15 mg) in benzene (7 ml) was added anhydrous K$_2$CO$_3$ (100 mg), methyl vinyl ketone (50 µl), and 18-crown-6 (one crystal). The mixture was refluxed under N$_2$ for 24 h after which it was filtered and the filtrate was concentrated in-vacuo. Preparative TLC of the residue (silica gel; acetic acid/methanol/chloroform, 1:5:94) gave 2-(3-oxobutyl)garveatin A (140) (12 mg) and unreacted garveatin A (2 mg). Compound 140: obtained as a yellow oil. R$_f$ 0.39; UV (MeOH) neutral 231, 271, 415 nm, basic 240, 268, 420 nm; $^1$H NMR (CDCl$_3$, 400 MHz) 1.48(s, 3H), 1.54(s, 3H), 1.58(s, 3H), 2.04(s, 3H), 2.24(m, 4H), 2.34(s, 3H), 2.55(s, 3H), 6.65(s, 1H), 7.12(s, 1H), 10.13(s, 1H), 16.29(s, 1H); $^{13}$C NMR (CDCl$_3$) 20.7, 24.1, 27.1, 29.9, 30.7, 31.3, 32.3, 38.9, 47.9, 58.0, 107.8, 110.8, 115.5, 120.2, 125.3, 139.5, 141.2, 142.5, 156.0, 166.1, 204.1, 204.2, 206.9, 211.3 ppm; MS m/z (rel. intensity) 410 (M$^+$ 18), 341 (16), 340 (32), 326 (25), 325 (100), 310 (9), 297 (12).

Garvalone B (141): obtained as a light yellow oil (3:2 mixture of diastereomers). R$_f$ 0.44; [α]$_D$ +136.92° (c 0.39, CHCl$_3$); UV (MeOH) 236, 290, 385 nm; $^1$H NMR (300 MHz, CDCl$_3$) 1.464, 1.470(s, 3H), 1.566, 1.533(s, 3H), 1.584, 1.611(s, 3H), 1.646(d, J=7 Hz, 3H), 2.063, 2.042(s, 3H), 2.234(m, 4H), 3.419, 3.424 (dd, J=18, 12 Hz, 1H), 4.387 (dd, J=18, 3 Hz, 1H), 4.732(m, 1H), 7.060(s, 1H), 7.102(s, 1H), 11.503(s, 1H), 15.442(s, 1H) ppm; HRMS 438.1680, calc'd for C$_{25}$H$_{26}$O$_7$, 438.1679; LRMS m/z (rel. intensity) 438 (M$^+$, 12), 368(10), 353(5), 325(3).
Garvalone B diacetate (142): \textsuperscript{1}H NMR (300MHz, CDCl\textsubscript{3}) 2.40(s, 3H), 2.47(s, 3H), 7.53(s, 1H), 7.75(s, 1H) ppm.

**Synthesis of garvalone B (141):**

Garvin B (126) (15 mg) was dissolved in benzene (8 ml) containing 100 mg of K\textsubscript{2}CO\textsubscript{3} (anhyd.) and a crystal of 18-crown-6. Excess methyl vinyl ketone (100 ul) was added and the mixture was stirred at reflux overnight, under N\textsubscript{2}. The reaction mixture was then filtered and the filtrate concentrated in-vacuo. Preparative TLC (5% MeOH/CHCl\textsubscript{3}) purification gave garvalone B (141) (8 mg) as a 2:3 mixture of diastereomers and unreacted garvin B (4 mg).

**Annulin A (144):**

Obtained as orange crystals from 95% ethanol, mp 174-176\textdegree C; R\textsubscript{f} 0.51; UV (MeOH) neutral 217, 247 439nm, basic 286, 559nm; IR (CHCl\textsubscript{3}) 1749.9, 1657.2, 1616.1 \textpercm; \textsuperscript{1}H NMR (300 MHz,CDCl\textsubscript{3}) 1.14(t,3H,J=7Hz), 1.63(s,3H), 1.72(s,3H), 2.44(s,3H), 2.76(q,2H,J=7Hz), 3.87(s,3H), 4.92(br s,1H), 7.47(s,1H), 12.12(s,1H) ppm; \textsuperscript{13}C NMR (75 MHz,CDCl\textsubscript{3}) 185.97, 180.92, 169.35, 160.32, 154.29, 145.63, 140.76, 139.81, 130.37, 122.23, 122.10, 113.40, 101.45, 89.08, 54.04, 27.94, 26.43, 20.09, 19.50, 12.73 ppm; HRMS 360.1221, calcd. for C\textsubscript{19}H\textsubscript{20}O\textsubscript{7}: 360.1209; LRMS m/z (rel. intensity) 360(M\textsuperscript{+},6), 342(2), 327(1), 301(45), 283(100), 255(14).

**Annulin B (148):**

Obtained as an orange oil; R\textsubscript{f} 0.51; [\textalpha]_D +8.0\textdegree (c.0.2,CHCl\textsubscript{3}); UV (MeOH) neutral 208, 255, 293, 425nm, basic 235, 270(sh), 307(sh), 530nm; IR (CHCl\textsubscript{3}) 1757, 1736, 1657, 1638.
$^{1}$H NMR (300 MHz, CDCl$_3$) 1.15(t, 3H, J=7Hz), 1.49(s, 3H), 1.51(s, 3H), 1.85(s, 3H), 2.42(s, 3H), 2.73(q, 2H, J=7Hz), 3.76(s, 3H), 7.31(s, 1H), 12.35(s, 1H) ppm; $^{13}$C NMR (75 MHz, CDCl$_3$) 203.02(s), 181.14(s), 178.62(s), 167.68(s), 163.79(s), 160.43(s), 147.64(s), 136.06(s), 127.59(s), 120.65(d), 119.11(s), 111.11(s), 84.48(s), 53.45(q), 43.64(s), 25.90(q), 23.78(q), 20.40(q), 20.38(q), 19.10(t), 12.77(q) ppm; HRMS 386.1361, calc'd. for C$_{21}$H$_{22}$O$_7$: 386.1366; LRMS m/z (rel. intensity) 386(M$^+$_17), 358(25), 343(100), 283(17).

Antimicrobial assays.

A standard in-vitro disc (0.25 in.) bioassay was used to the antibacterial and antifungal activity of the G. annulata metabolites. Activities are reported as minimum inhibitory concentrations (MIC) in µg/disc.


(c) *ibid.*, Volume 3, (1980).


(e) *ibid.*, Volume 1, (1978).


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