SECONDARY METABOLITES FROM SELECTED
BRITISH COLUMBIAN MARINE ORGANISMS

By

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We accept this thesis as conforming
to the required standard

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Date April 30, 1987
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The two purine alkaloids, phidolopin (36) and desmethylphidolopin (37), originally isolated from *Phidolopora pacifica*, were synthesized in order to produce sufficient quantities of the natural products for extended biological and pharmacological screening and to confirm the previous structural assignment of 37 which was based on spectral data.

Various combinations of phidolopin (36), desmethylphidolopin (37), 4-hydroxymethyl-2-nitrophenol (38) and 4-methoxymethyl-2-nitrophenol (39) were isolated from four different species of bryozoans, *Diaperoecia californica*, *Heteropora alaskensis*, *Tricellaria ternata* and *Hippodiplosia insculpta*. A dietary origin is suggested for these metabolites.

The red sponge, *Anthoarcuata graceae*, yielded six novel steroids including the Δ⁴-3,6-diketosteroids 116, 117, the A-nor steroids anthosterone A (118) and anthosterone B (119) as well as two diosphenol containing steroids, 120 and 121. The proposed structures were based on a combination of spectral analysis, chemical interconversions, synthesis, and single crystal X-ray diffraction analysis.
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### Abbreviations

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<tr>
<td>CDCl₃</td>
<td>Chloroform-d₁</td>
</tr>
<tr>
<td>CHCl₃</td>
<td>Chloroform</td>
</tr>
<tr>
<td>DMSO</td>
<td>Dimethylsulfoxide</td>
</tr>
<tr>
<td>EtOAc</td>
<td>Ethyl acetate</td>
</tr>
<tr>
<td>g</td>
<td>Grease peak</td>
</tr>
<tr>
<td>HPLC</td>
<td>High performance liquid chromatography</td>
</tr>
<tr>
<td>HRMS</td>
<td>High resolution mass spectrum</td>
</tr>
<tr>
<td>IR</td>
<td>Infrared</td>
</tr>
<tr>
<td>MS</td>
<td>Low resolution mass spectrum</td>
</tr>
<tr>
<td>Na₂SO₄</td>
<td>Sodium sulfate (anhydrous)</td>
</tr>
<tr>
<td>¹H NMR</td>
<td>Proton nuclear magnetic resonance</td>
</tr>
<tr>
<td>¹³C NMR</td>
<td>Carbon-13 nuclear magnetic resonance</td>
</tr>
<tr>
<td>NOE</td>
<td>Nuclear Overhauser enhancement</td>
</tr>
<tr>
<td>mp</td>
<td>Melting point</td>
</tr>
<tr>
<td>RT</td>
<td>Room temperature</td>
</tr>
<tr>
<td>S</td>
<td>Solvent signal</td>
</tr>
<tr>
<td>SCUBA</td>
<td>Self contained underwater breathing apparatus</td>
</tr>
<tr>
<td>TLC</td>
<td>Thin layer chromatography</td>
</tr>
<tr>
<td>U</td>
<td>Unknown impurity signal</td>
</tr>
<tr>
<td>UV</td>
<td>Ultraviolet</td>
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Abbreviations for multiplicities of NMR signals:

s = singlet

d = doublet

t = triplet

q = quartet

dd = doublet or doublets

bs = broad singlet

m = multiplet

This thesis has been written in conformance with the "Handbook for Authors", published by the American Chemical Society; Washington, D.C., 1978.
A. INTRODUCTION TO THE BRYOZOANS

Bryozoans, commonly found in abundance in predator-rich, competitive environments, have demonstrated the ability to grow on all forms of hard surface ecological space such as rocks, shells, corals, wood and steel. The adaptability of bryozoans has made them one of the most prevalent groups of fouling organisms. For example, some 130 species have been taken from ship bottoms, where they show a general resistance to anti fouling paints.\(^1\) Their remarkable adhesion properties have led to full scale studies on their secretory products in the hope of finding viable new bioadhesive materials acceptable for clinical use by the dental and medical professions.\(^2\)

Of particular interest, however, are the chemical studies conducted on marine bryozoans in the past twenty years which have yielded a number of novel biologically-active secondary metabolites. These findings have prompted continuing chemical studies of members of this phylum which have as their goals the discovery of new biologically-active compounds and answers regarding the origin of the isolated metabolites.

i. Biology

The phylum Bryozoa (or polyzoa or Ectoprocta) contains approximately 4000 known living species. The phylum is divided into three classes, Phylactolaemata, Gymnolaemata, and Stenolaemata. The classes
Stenolaemata and Gymnolaemata include only marine bryozoans while the class Phylactolaemata is restricted to approximately 50 known fresh water species.

Bryozoans are colonial filter feeding animals which vary in height and width and occur in a variety of morphological forms. Some of the names used to describe these forms are false-corals, sea-mats, and moss-animals. In British Columbia all of these forms are common. Some examples include: *Heteropora pacifica* - coral like, *Bugula sp.* - moss- animal type, and *Membranipora membranacea* - sea-mat type.

Bryozoan colonies are built of a replicated series of individual zooids which have body walls which are calcareous, gelatinous or chitinous and usually about 0.5 mm in length. Individuals of most species are encased in a non-living envelopment that contains an opening (orifice) for the protrusion of the circular or horse-shoe shaped lophophore used to gather the small plankton (chiefly diatoms and other phytoplankton) that make up the bryozoans' diet. The interior of the body is occupied largely by the spacious coelom, a U shaped digestive tract, muscles and the anus which opens alongside the lophophore. The zooids of a colony are attached through pores or gaps in the body wall. Some zooids in a colony are modified for specialized functions (polymorphism) such as feeding (autozooid, the largest zooid), cleaning, protection or brooding of the young (heterozooids).

Bryozoan colonies are hermaphroditic with both male and female zooids occurring in the same colony. In most species, the fertilized egg will pass into a brooding chamber producing larvae which are non-feeding. The larvae have a very brief planktonic existence before
settling on a hard surface. Non brooding bryozoans possess larvae with a functional digestive tract which can feed during the several months of larval life prior to settling. After settlement, the larvae begins to transform into a zooid within hours.

ii) Natural Products Chemistry

The biological and ecological aspects of bryozoans have been exhaustively investigated, however, until recent years very little work had been conducted on the chemistry of bryozoans. The first modern chemical and pharmacological studies of marine invertebrates were carried out around 1960, with extracts from sponges and coelenterates showing some interesting antibiotic activity. In 1970, Pettit and Day reported the first evidence of antineoplastic activity in extracts of marine invertebrates. Roughly 9-10% of the organisms tested displayed a significant level of activity against the U.S. National Cancer Institutes (NCI) murine P388 lymphocytic leukemia (PS system) or Walker carcinosaoma 256 test lines in the rat. Among the organisms tested by Pettit et al. was Bugula neritina (bryozoa), collected off the Northeast coast of the Gulf of Mexico.

The anticancer studies initiated in 1968 by Pettit et al. on B. neritina culminated in 1982 with the isolation and structure elucidation of a remarkable antineoplastic compound named bryostatin 1 (1). The structure of this compound, which was the first pure metabolite isolated from a bryozoan, was solved by a combination of x-ray crystallographic
and spectroscopic techniques. Bryostatin 1 (1) was active against the murine P388 lymphocytic leukemia (PS system) both in vivo and in vitro. Subsequent studies on the sea-mat like *B. neritina* have yielded four structural variants on the same bryopyran macrolide skeleton (1a) found in bryostatin 1 (1). They are bryostatins 2 (2), 3 (3), 4 (4), 5 (5), 6 (6) and 7 (6a).
R = COCH₃
R' = \text{\text{\textbackslash greek\text{\textbackslash text{nu}}} vinyl}
R'' = H
3
A simultaneous study by Pettit et al. on *Amanthia convoluta* (bryozoan) also yielded an extract possessing antineoplastic activity. *A. convoluta*, which was found to grow together with *B. neritina* in a parasitic or epiphytic-like manner, has yielded the same group of bryostatins (1-6) as well as a new compound designated bryostatin 8 (7). It is unclear whether brystatin 8 is a genuine constituent of *A. convoluta* or transferred from *B. neritina* and concentrated by *A. convoluta*.
Still to be determined is whether these compounds originate from de novo biosynthesis or from a common food source such as bacteria or phytoplankton. Pettit has postulated that the isolation of bryostatin 4 (4) from \textit{B. neritina} collected in such diverse geographical areas as the Gulf of Mexico and the Gulf of Sagami, Japan indicates that the macrocyclic lactones are biosynthetic products rather than of dietary origin. However, this can only be answered by finding a dietary source or by biosynthetic $^{14}$C-acetate feeding studies.\textsuperscript{13}

Christophersen and Carle, in a series of articles starting in 1978 reported the isolation of monoterpenes and nine new bromo-alkaloids from the bryozoan \textit{Flustra foliacea}. Their work with marine bryozoa was initiated by an interest in the study of chemical messengers.\textsuperscript{14} In a prior study carried out by Al-Ogily and Knight-Jones\textsuperscript{15} on marine 'transmitters', it was reported that older fronds of \textit{F. foliacea} emitted a lemonlike odor. This odor was attributed to the monoterpane constituents of the bryozoan, namely, cis-citral (8), trans-citral (9), citronellol (10), nerol (11) and geraniol (12).\textsuperscript{16}
The nine novel bromo-alkaloids isolated from *F. foliacea* possess either the physostigmine, indole, or quinoline ring systems. All the structures were solved by spectroscopic means. In 1979, Christophersen and Carle reported the isolation and structure elucidation of flustramine A (13) and flustramine B (14), both possessing the unprecedented bromophysostigmine skeleton and a rare N-8 γ,γ-dimethylallyl substituent.\(^1\)

\[
\begin{align*}
\text{Br} & \quad \text{N} \quad \text{N} \\
\text{H} & \quad \text{N} \\
\text{CH}_3 & \quad \text{CH}_3
\end{align*}
\]

**13** \[
\begin{align*}
\text{Br} & \quad \text{N} \quad \text{N} \\
\text{H} & \quad \text{N} \\
\text{CH}_3 & \quad \text{CH}_3
\end{align*}
\]

**14**

Christophersen and Carle, in a continuation of the study of *F. foliacea* (L.), reported the isolation and structure elucidation of flustramine C (15), flustraminol A (16) and flustraminol B (17).\(^2\)

These three structures possess the basic 6-bromo physostigmine skeleton, however, in contrast to compounds (13) and (14) they all contain only one isoprene substituent. In 1981, Wulf et al. reported the isolation
and structure elucidation of a bromo-indole derivative, flustrabromine (18). Additional *F. foliacea* metabolites include flustramide A (19), 6-bromo-N-methyl-N-formyltryptamine (20) and 7-bromo-4-(2-ethoxyethyl)quinoline (21), the first isolated naturally occurring bromoquinoline. A series of new strongly antimicrobial bromoalkaloids, dihydroflustramine C (22), flustramine D (23), dihydroflustramine C N-oxide (24), flustramine D N-oxide (25) and isoflustramine D (26), were recently isolated from *Flustra foliacea* collected in the Bay of Fundy.

Chartelline A (27), a novel pentahalogenated alkaloid from the bryozoan *Chartella papyracea*, which belongs to the same family as *F. foliacea*, was reported in 1985 by Chevolot et al. The structure of chartelline A (27) was unambiguously assigned by single crystal X-ray crystallography.
Two gramine derived bromo-alkaloids, 2,5,6-tribromo-N-methylgramine (28) and its N-oxide (29), were isolated by Sato and Fenical in 1983 from the subtropical bryozoan Zoobotryon verticilatum. Alkaloid N-oxides are commonly found along with free alkaloids from terrestrial sources, however, their occurrence in F. foliacea and Z. verticilatum.

![Image of chemical structures](image-url)
appear to be the only such reported examples from marine sources. Initial bioassays show that compound (28) inhibits cell division of fertilized sea urchin eggs (ED$_{50}$ = 16 ug/mL). Compound (28) was synthesized by an Italian group in 1977.\textsuperscript{25}

(2-Hydroxyethyl)dimethylsulfonium ion (30), has been determined to be the causative agent of Dogger Bank itch, an eczematous allergic contact dermatitis caused by exposure to the bryozoan *Alcyonidium gelatinosum*.\textsuperscript{26} Sulfoxonium ions had not previously been found in nature.

\[
\begin{align*}
&\text{CH}_3\text{CH}_2\text{-CH}_2\text{-OH} \\
&\text{CH}_3\text{CH}_2\text{-OH} \\
&\text{CH}_3\text{CH}_2\text{-OH}
\end{align*}
\]

\textbf{30}

Nudibranchs are one of the main bryozoan predators. Methanolic extracts of three nudibranchs, *Roboastra tigris*, *Tambje eliora* and *Tambje abdere*, have all been found to contain the same group of biologically active bipyroles, tambjamines A-D (31-34).\textsuperscript{27} These compounds were all traced to a dietary source, the bryozoan *Sessibugula translucens*. Tambjamines A-D are the major secondary metabolites of *S. translucens* and it is believed the nudibranchs use these dietary compounds for self defence. When *R. tigris*, the large carnivorous
nembrothid nudibranch attacks the two smaller nembrothid nudibranchs *T. abdere* and *T. eliora* the following occurs; *T. abdere* produces a yellow mucus from goblet cells in the skin which causes *R. tigris* to break off the attack. The secretion has been shown to contain mainly tambjamines A-D. *T. eliora* does not appear to produce a defensive secretion, however, it did attempt to escape *R. tigris* by a vigorous writhing motion. In subsequent laboratory observations it has been
shown that *R. tigris* preferred to eat *T. eliopa* rather than *T. abdere*. In further bioassay studies, these bipyrrroles have displayed antimicrobial activity against various bacteria.

Fusetani et al.\(^{28}\) recently reported the isolation of a blue antimicrobial tetrapyrrole 35 from the bryozoan *Bugula dentata*. Compound 35 was previously found in a mutant strain of the bacteria *Serratia marcescens*.

\[\text{35} \]

A new purine derivative, phidolopin (36), was reported in 1984 by Ayer and Andersen\(^{29}\) from the bryozoan *Phidolopora pacifica* collected in the waters off British Columbia. Co-occurring with this compound was its desmethyl derivative (37), as well as two nitrophenols, 4-hydroxy-methyl-2-nitrophenol (38) and 4-methoxymethyl-2-nitrophenol (39). Phidolopin was shown to possess potent in-vitro antimicrobial and antialgal activities.
B. SYNTHESIS OF PHIDOLOPIN AND DESMETHYLPHIDOLOPIN

The total synthesis of phidolopin (36) and desmethylphidolopin (37) was undertaken in order to confirm the structure of 37 proposed by Ayer et al. \(^9\) in their 1984 study of the chemistry of \(P.\) pacifica, and also to devise an efficient method for producing sufficient quantities of both metabolites for further biological testing.

![Chemical structures of 36 and 37]

The synthetic plan for the total synthesis of these two compounds is shown in Scheme 1. The first step of the synthesis entailed the protection of the hydroxyl functionality on 4-methyl-2-nitrophenol (40) with a base stable protecting group, which could be easily removed as the final step in synthesis. Protecting the phenol as its methyl ether or as its methoxymethyl ether (MOM) appeared to be the most direct approach.

The following methods were attempted to accomplish the desired protection in the greatest yield (Scheme 2). Methylation of 4-methyl-2-
Scheme 1: Synthetic plan for phidolopin (36) and desmethyl-philodolopin (37)

\[
\begin{align*}
40 \quad \text{OH} & \quad \text{NO}_2 \\
\text{OP} & \quad \text{NO}_2 \\
& \quad \text{P=Me, MOM} \\
\downarrow & \\
\text{OP} & \quad \text{NO}_2 \\
& \quad \text{Br} \\
& \quad R=\text{Me, H} \\
\end{align*}
\]

\[
\begin{align*}
36 & \quad R=\text{CH}_3 \\
37 & \quad R=\text{H}
\end{align*}
\]
nitrophenol (40) was achieved by reacting 40 in acetone and excess potassium carbonate with 1.5 equivalents of methyl iodide under reflux (Scheme 2a). Continuous monitoring by thin layer chromatography (TLC) (1:10 ethyl acetate/petroleum ether) showed rapid formation of a new UV absorbing component at $R_F$ 0.13 which was more polar than the starting material at $R_F$ 0.35. After 2.5 hr, it appeared by tlc that the reaction
had proceeded to completion. Work-up of the reaction mixture by partitioning between distilled water and dichloromethane, drying of the organic layer with Na$_2$SO$_4$, filtering and evaporation in vacuo yielded a heavy brown oil. Purification of this oil by preparative TLC (2:10 ethyl acetate/petroleum ether) gave 41 as a viscous yellow oil (yield 71%). $^1$H NMR (Figure 1) and mass spectrometric analysis of this oil showed the required $^1$H NMR resonance for the OCH$_3$ protons at $\delta$ 3.95 ppm (s, 3H), (Table 1) and a MS parent ion at m/z 167 (Scheme 3).

Two methods, both involving the use of chloromethyl methyl ether, but using two different bases, were explored as approaches to the preparation of the -OCH$_2$CH$_3$ (MOM) derivative (Scheme 2b, c).$^{31,32}$ In the first case, the phenoxide of nitrophenol (40) was prepared in dry diethyl ether using a suspension of sodium hydride (excess). ChloromethyImethylether (1.5 equiv) was added dropwise at 0°C to the red solution of the phenoxide. The reaction, which was continually monitored by TLC (1:10 ethyl acetate/hexanes), was allowed to proceed at room temperature for 48 hr. A new more polar UV absorbing component (R$_f$ 0.17) was formed. The work-up involved partitioning the reaction mixture between water and diethyl ether, drying the organic layer over Na$_2$SO$_4$, filtering and evaporation in vacuo. Purification by preparative TLC (1:10 ethyl acetate/hexanes) yielded 42 as a clear yellow oil (yield 36.3%). The $^1$H NMR of 42 (Figure 2) showed resonances at $\delta$ 3.53 ppm (s, 3H), and $\delta$ 5.25 ppm (s, 2H) assigned to the MOM group (Table 1). A EIMS of 42 showed a parent ion at m/z 197 as required (Scheme 4). A large amount of unreacted starting material 40 was also recovered from this reaction.
Figure 1: 80 MHz $^1$H NMR of compound 41
Figure 2: 80 MHz $^{1}$H NMR of compound 42
Table 1: 80 MHz $^1$H NMR data on protected nitrophenols 41 and 42

<table>
<thead>
<tr>
<th>H on C#</th>
<th>Chemical shift, δ ppm (CDCl$_3$)</th>
<th>41</th>
<th>42</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>7.65 (d, J = 2 Hz, 1H)</td>
<td>7.56 (d, J = 2 Hz, 1H)</td>
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<tr>
<td>5</td>
<td>7.32 (dd, J = 9, 2 Hz, 1H)</td>
<td>7.30 (dd, J = 9, 2 Hz, 1H)</td>
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</tr>
<tr>
<td>6</td>
<td>6.97 (d, J = 8 Hz, 1H)</td>
<td>7.13 (d, J = 9 Hz, 1H)</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>2.35 (s, 3H)</td>
<td>2.35 (s, 3H)</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>3.95 (s, 3H)</td>
<td>5.25 (s, 2H)</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td></td>
<td>3.53 (s, 3H)</td>
<td></td>
</tr>
</tbody>
</table>

Chemical structures of 41 and 42:
Scheme 3: Interpretation of the MS fragmentation of 41

\[
\begin{align*}
\text{41} \quad \begin{array}{c}
\text{OCH}_3 \\
\text{NO}_2
\end{array} \\
\rightarrow \quad \begin{array}{c}
m/z 167 (78\%) \\
\text{m/z 167 (27\%)} \quad \text{-NO}_2, \text{-CH}_3 \\
\text{m/z 137 (49\%)} \\
\text{m/z 120 (82\%)} \\
\end{array}
\end{align*}
\]

Scheme 4: Interpretation of the MS fragmentation of 42

\[
\begin{align*}
\text{42} \quad \begin{array}{c}
\text{OCH}_2\text{OCH}_3 \\
\text{NO}_2
\end{array} \\
\rightarrow \quad \begin{array}{c}
m/z 197 (55\%) \\
\text{m/z 167 (37\%)} \quad \text{-NO}_2, \text{-CH}_3 \\
\text{m/z 166 (3\%)} \quad \text{-OCH}_3
\end{array}
\end{align*}
\]
In an attempt to devise a higher yielding and cleaner method for the preparation of the MOM derivative 42, we modified the reaction conditions to use the milder base, potassium carbonate. Thus, excess potassium carbonate and 6 equivalents of chloromethyl methyl ether were reacted with the nitrophenol 40 in acetone at room temperature. Under these conditions, the reaction went almost spontaneously upon addition of the chloromethyl methyl ether as indicated by TLC (1:10 ethyl acetate/hexanes). There appeared to be much less starting material after 10 to 15 minutes than there was in the previous method after 48 hr. Following work-up and purification conducted as before, a single component (yield 73%) was isolated, which had identical spectral features to the previously prepared MOM protected nitrophenol 42.

Lister et al. have looked at the optimization of conditions for production of 7-benzylxanthine (43) and 9-benzylxanthine (44) derivatives via the N7 and N9 alkylation of xanthines.

They found that 7-(4-nitrobenzyl)theophylline (48) or 7-(4-methylbenzyl)theophylline (50) could be produced by refluxing 4-nitrobenzyl-
bromide (47) or 4-methylbenzylbromide (49) with theophylline (45) in aqueous sodium hydroxide (.1 M) for 2 hours (Schemes 5 and 6). Using Lister's work as a model, it seemed that a logical next step in the synthesis of phidolopin (36) and desmethylphidolopin (37) was the conversion of the protected nitrophenols 41 and 42 to benzyl bromide derivatives which could act as alkylating agents.

Bromination of 4-methoxy-3-nitrotoluene (41) was successfully carried out with N-bromosuccinimide (NBS) as the brominating agent (Scheme 7).34

The reaction was carried out by dissolving compound 41 and NBS (2 equiv) in a minimum amount of distilled carbon tetrachloride and then refluxing this solution in the presence of light (150 W tungsten bulb).

Scheme 5: Alkylation of theophylline (45) with 47
Scheme 6: Alkylation of theophylline with 49

\[ \text{45} + \text{49} \xrightarrow{\text{Base}} \text{50} \]

Scheme 7: Bromination of protected nitrophenol 41

\[ \text{41} \xrightarrow{\text{NBS/CCl}_4, h\nu, reflux} \text{51} \]
The reaction was monitored very closely by TLC (3:10 ethyl acetate/hexanes) to ensure that only monobromination occurred. After only 5 minutes, a new more polar component became apparent on TLC. As time proceeded, the reaction mixture went from yellow to a reddish brown, perhaps indicating the formation of bromine. When a sufficient amount of the new component had formed, the reaction was halted and carefully worked up. Rapid purification of the new component by preparative TLC (3:10 ethyl acetate/hexanes) in a darkened room, afforded a yellow oil (yield 45%), which was identified by low resolution mass spectrometry to be the desired benzyl bromide 51 (Scheme 8). $^1$H NMR was not useful in the characterization of 51 due to the rapid decomposition of this highly labile substance.

Scheme 8: MS fragmentation of compound 51

\[ \text{m/z 120 (25\%)} \]
\[ \text{m/z 166 (65\%)} \]
\[ \text{m/z 245/247 (2.3\%)} \]
The bromination of the MOM protected nitrophenol 42 was carried out in a slightly modified manner (Scheme 9). Compound 42 was dissolved in a minimum amount of carbon tetrachloride and the solution was brought to reflux while being irradiated with a 220 W sun lamp. Two equivalents of NBS in carbon tetrachloride were slowly added to the refluxing solution. The reaction was carefully monitored by TLC (3:10 ethyl acetate/hexanes) to ensure that only monobromination occurred.

Scheme 9: Bromination of MOM protected nitrophenol 42

\[
\text{OMOM} \quad \begin{array}{c}
\text{NBS/CCl}_4 \\
\text{hv, reflux}
\end{array} \quad \begin{array}{c}
\text{OMOM} \\
\text{Br}
\end{array}
\]

When the reaction was left to run for more than 20 minutes, the formation of polybrominated species would occur as indicated by the appearance of numerous more polar TLC spots. The monobrominated product 52 was visible on TLC within 5 minutes and the reaction was complete by 15 minutes. The reaction was terminated by removing the light source. To avoid the loss of material due to decomposition, the brominated species was never isolated for characterization, but instead it was used directly in the alkylation reactions. Even though 52 was not character-
ized spectroscopically, TLC showed 52 to be slightly more polar (Rf 0.28) than the starting material 42 (Rf 0.34) (3:10 ethyl acetate/hexanes) similar to the polarity differences between 41 and 51.

Alkylation was carried out with both the methyl as well as the MOM protected alkylating agents. Reaction of 51 with theophylline in 1.5 mL of refluxing 0.1 M sodium hydroxide and 3 mL tetrahydrofuran for 2 hr gave both the desired N7 alkylated compound 53 as well as the N9 alkylated compound 54 (Scheme 10). The N7 and N9 alkylated products could be distinguished by examination of the 1H NMR spectra of the two compounds (Table 2). The chemical shift of the benzylic protons in the

Scheme 10: Alkylation of theophylline (45) with 51
Table 2: 80 MHz $^1$H NMR data comparison of 53 and 54

<table>
<thead>
<tr>
<th>H on C or N#</th>
<th>Chemical shift, δ ppm</th>
<th>53</th>
<th>54</th>
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</thead>
<tbody>
<tr>
<td>1'</td>
<td>5.48 (s, 2H)</td>
<td>5.53 (s, 2H)</td>
<td></td>
</tr>
<tr>
<td>N1 Me</td>
<td>3.43 (s, 3H)</td>
<td>3.39 (s, 3H)</td>
<td></td>
</tr>
<tr>
<td>N3 Me</td>
<td>3.58 (s, 3H)</td>
<td>3.56 (s, 3H)</td>
<td></td>
</tr>
<tr>
<td>3'</td>
<td>7.38 (dd, J = 9, 2 Hz, 1H)</td>
<td>7.58 (dd, J = 9, 2 Hz, 1H)</td>
<td></td>
</tr>
<tr>
<td>4'</td>
<td>7.00 (d, J = 9 Hz, 1H)</td>
<td>7.09 (d, J = 9 Hz, 1H)</td>
<td></td>
</tr>
<tr>
<td>7'</td>
<td>7.68 (d, J = 2 Hz, 1H)</td>
<td>7.89 (d, J = 2 Hz, 1H)</td>
<td></td>
</tr>
</tbody>
</table>

![Diagram of 53 and 54](image)
N7 alkylated species was found to be δ 5.48 ppm (s, 2H), whereas the benzylic protons in the N9 alkylated compound resonated at δ 5.53 ppm (s, 2H). Also altered were the shifts of the aromatic protons as seen in Table 2. Comparison of the chemical shifts of the benzylic protons to the observed values for phidolopin (36) allowed the assignment of the correct structures.

In the case of the MOM derivative 42, the bromination and alkylation reactions were carried out without isolation of intermediate 52 (Scheme 11). Following the bromination reaction, the reaction mixture was briefly cooled and the succinamide was filtered off.

Scheme 11: Bromination and alkylation

\[
\begin{align*}
\text{42} & \xrightarrow{\text{NBS}} \text{52} \\
\text{OMOM} & \quad \text{OMOM} \\
\text{Br} & \quad \text{R} \\
\text{NO}_2 & \quad \text{NO}_2 \\
\end{align*}
\]

55 \( R=\text{CH}_3 \)  
56 \( R=\text{H} \)
The resulting filtrate was carefully evaporated in vacuo to near dryness before being dissolved in THF and added dropwise to a solution of theophylline (45) in 0.1 M sodium hydroxide solution which had been stirring for 20 minutes at room temperature. The amount of theophylline used in the alkylation reaction assumed a 100% yield in the bromination reaction. As a result, excess theophylline (45) always appeared in the reaction mixture. After 18 hours of stirring at room temperature, TLC analysis showed the presence of the protected nitrophenol 42 as well as a new more polar component. Work-up and purification by preparative TLC (3:10 ethyl acetate/hexanes) yielded the polar constituent as a white solid (yield 25%) which was shown by $^1$H NMR (Figure 3) and mass spectrometric analyses to be the desired protected phidolopin 54. Key features of the $^1$H NMR spectrum were a sharp two proton singlet at $\delta$ 5.46 ppm assigned to benzylic protons and resonances at $\delta$ 3.51 and 5.25 ppm assigned to the MOM group. The mass spectrum of 55 showed a parent ion at m/z 375 (8% of base) (Scheme 12). The yield in this two step reaction (32%) proved to be the lowest in the total synthesis, however, nearly 50% of the protected nitrophenol 42 was recoverable by chromatographic means after work-up of the reaction.

Using the same method of bromination and alkylation, MOM protected desmethylphidolopin (56) was synthesized in nearly the same yield (24.7%) by substituting 3-methylxanthine (46) for theophylline (45) (Scheme 11). As before, only the N7 product was formed, with no evidence of N1 or N9 alkylation (Figure 4). Lister found that exclusive N9 alkylation of xanthines could be achieved by carrying out the alkylation in a more concentrated basic solution under reflux conditions for 3
Figure 3: 80 MHz 1H NMR of MOM protected desmethylphospholipin 55
Figure 4: 80 MHz $^1$H NMR of MOM protected desmethylphidolopin 56
hours while C-8 alkylation would occur in dimethylformamide under reflux for 4 hours (Scheme 13). It appears that the N7 alkylation product is the kinetically preferred product while the N9 and C8 are the thermodynamically preferred alkylation products of theophylline. As seen previously in the alkylation of theophylline with the methylated nitrophenol 41, reflux conditions yielded a mixture of products (Scheme 9). Therefore, it appears the use of milder reaction conditions promotes the alkylation of only one position, N7.

Deprotection of the methylated phidolopin derivative 53 proved to be difficult. Using the method outlined by Olah et al.,\textsuperscript{36} which allows for the cleavage of methyl ethers by treatment with chlorotrimethyl-
Scheme 13: Alkylation of theophylline (45) under varied conditions

- silane and sodium iodide in dry acetonitrile at room temperature for 8 to 10 hours, yielded only starting material (Scheme 14).

Deprotection of the MOM derivatives proved to be much simpler and gave high yields of product. Refluxing either MOM derivative 55 or 56 for one hour in a minimum amount of chloroform containing 50% acetic acid\textsuperscript{37} plus one drop of concentrated sulphuric acid yielded phidolopin.
Scheme 14: Deprotection of 53 with chlorotrimethylsilane

\[
\begin{align*}
53 & \xrightarrow{TMSCl, KI, CH_3CN, \Delta} \text{N.R.}
\end{align*}
\]

(36) and desmethylphidolopin (37) respectively (Table 3) after purification by preparative TLC (Figures 5, 6) (Scheme 15).

Scheme 15: Deprotection of 55 and 56 with dilute acid

\[
\begin{align*}
55 \quad R = \text{CH}_3 \\
56 \quad R = \text{H}
\end{align*}
\]
Figure 5: 80 MHz $^1H$ NMR of phidolopin 36.
Figure 6: 80 MHz $^1$H NMR of desmethylphidolopin 37
Table 3: 80 MHz $^1H$ NMR data for phidolopin (36) and desmethyl-phidolopin (37)

<table>
<thead>
<tr>
<th>H on C or N$^#$</th>
<th>36</th>
<th>37</th>
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<tbody>
<tr>
<td>N1-H</td>
<td>-</td>
<td>11.03 (s, 1H)</td>
</tr>
<tr>
<td>N1 Me</td>
<td>3.59 (s, 3H)</td>
<td>-</td>
</tr>
<tr>
<td>N3 Me</td>
<td>3.59 (s, 3H)</td>
<td>3.46 (s, 3H)</td>
</tr>
<tr>
<td>8</td>
<td>7.65 (s, 1H)</td>
<td>7.82 (s, 1H)</td>
</tr>
<tr>
<td>1'</td>
<td>5.48 (s, 2H)</td>
<td>5.45 (s, 2H)</td>
</tr>
<tr>
<td>3'</td>
<td>7.63 (dd, J=1.9,8.5 Hz, 1H)</td>
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</tr>
<tr>
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<td>7'</td>
<td>8.06 (d, J = 1.9 Hz, 1H)</td>
<td>8.05 (d, J = 1.9 Hz, 1H)</td>
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</table>

![Chemical structures](36.png) ![Chemical structures](37.png)
Spectroscopic data (Table 3) obtained for the two synthetic products was identical to the spectroscopic data recorded for the natural products. The total synthesis of phidolopin (36) was reported by a Japanese group simultaneously with the completion of the total syntheses of phidolopin (36) and desmethylphidolopin (37) by this author in 1985. In addition to the synthesis of compound (36), assignment of the $^{13}$C NMR spectrum was reported (Table 4). Using this data and $^{13}$C NMR data of compound (36) (Figure 7), assignment of the spectrum of desmethylphidolopin (37) (Figure 8) was simplified (Table 4).
Table 4: $^{13}$C NMR data comparison for 36$^{38}$ and 37

<table>
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<tr>
<th>C#</th>
<th>36$^a$</th>
<th>37$^b$</th>
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<tr>
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<td>N3 Me</td>
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<td>7'</td>
<td>137.0 (d)</td>
<td>136.3 (d)</td>
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</tbody>
</table>

$^a$ 100 MHz, CDCl$_3$

$^b$ 75 MHz, CDCl$_3$ + DMSO-d$_6$
C. NITROPHENOLS FROM NORTHEAST PACIFIC BRYOZOANS

The isolation of phidolopin (36), from the bryozoan Phidolopora pacifica collected off the coast of British Columbia, was reported by Ayer et al.29 in 1984. Attention was drawn to this bryozoan by the lack of fouling organisms on its skeleton as well as by the strong in vitro anti-algal and anti-bacterial activity displayed by its crude extracts. Further investigation of P. pacifica (referred to as the "lacy bryozoan" due to its intricate calcium carbonate exoskeleton which resembles a ruffled lacy network) led to the isolation of other novel nitrophenols; desmethylphidolopin (37), 4-hydroxymethyl-2-nitrophenol (38) and 4-methoxymethyl-2-nitrophenol (39), the later believed to be an isolation artifact.

Encouraged by the discovery of interesting biologically active secondary metabolites in P. pacifica, we initiated an examination of other common Northeastern Pacific bryozoans which live in similar habitats with the hope of discovering additional new biologically active metabolites. All new bryozoan specimens were collected by hand using SCUBA. Crude methanol extracts of these animals were assayed for in-vitro antibacterial, antifungal and antialgal activity.

Diaperoecia californica (d'Orbigny 1852), usually referred to as the "Southern Staghorn" bryozoan, is commonly found on offshore reefs as well as intertidal rocks (-2 to -10 m) from the British Columbia coast to Baja, California. Classified in the bryozoan order Cyclostomata, D. californica possesses an inflexible calcium carbonate exoskeleton which
is coral-like in appearance, with each colony attaining a height of approximately 10 cm and a diameter of 13 to 15 cm. It is also identified by its light yellow tubular branches which have a flat cross-section.

*D. californica* was first collected in June 1984 in Barkley Sound, B.C. The bryozoans (653 g dried weight after extraction) were immediately soaked in methanol and refrigerated (-2°C) for up to seven days before work-up. At the end of this time, the methanol layer was decanted off and the bryozoans were ground in a Waring blender with
fresh methanol, and filtered. The combined reddish-brown methanol extracts were concentrated in vacuo down to about one third the original volume and the resulting aqueous methanolic layer was extracted with ethyl acetate. The red-brown ethyl acetate soluble extract was dried over anhydrous sodium sulfate. The sodium sulfate was filtered off and the ethyl acetate was evaporated in vacuo to yield 5.8 g (.89%) of a dark red oil. Flash chromatography gave three fractions distinguishable by similar chromatographic polarities, each of which on bioassay analysis afforded moderate antifungal and antibacterial activity. Purification of the most polar fraction by column chromatography on Sephadex LH-20 (7:3 methanol/chloroform) gave a strongly retained yellow band. TLC analysis of this band indicated a single component with an Rf value similar to phidolopin (36), which strongly absorbed both short wave (dark) and long wave (black) UV light. Analysis by $^1$H NMR (Table 5) as well as mass spectrometry (Scheme 16) confirmed the presence of phidolopin (36) (1.0 mg .001%), as yellow needles (mpt. 211-212).

Purification of the two less polar fractions by column chromatography on Sephadex LH-20 (7:3 methanol/chloroform) gave desmethylphidolopin (37) (3.7 mg, .001%) as a yellow solid (Table 5) (Scheme 17), as well as 4-hydroxymethyl-2-nitrophenol (38) (4.5 mg, .007%) as a yellow oil (Table 6) (Scheme 18). The $^1$H NMR and mass spectra of the compounds were identical to the spectra of authentic materials.

This general isolation and purification procedure was employed in the examination of three other bryozoans, namely, Heteropora alaskensis, Hippodiplosia insculpta, and Triccelaria ternata with the results tabulated in Table 13 (Section 6). $^1$H NMR data (Figures 5, 6, 9, 10) as
well as MS fragmentation patterns for the isolated metabolites can be observed in Tables 5 and 6 and Schemes 16 and 19 respectively.

### Table 5: $^1$H NMR data for isolated nitrophenols 36-37

<table>
<thead>
<tr>
<th>H on C or N#</th>
<th>Chemical shift, $\delta$ ppm</th>
<th>$^{36a}$</th>
<th>$^{37a}$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td></td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>N1 Me</td>
<td>3.40 (s, 3H)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N3 Me</td>
<td>3.59 (s, 3H)</td>
<td>3.51 (s, 3H)</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>7.63 (s, 1H)</td>
<td>7.63 (s, 1H)</td>
<td></td>
</tr>
<tr>
<td>1'</td>
<td>5.46 (s, 2H)</td>
<td>5.39 (s, 2H)</td>
<td></td>
</tr>
<tr>
<td>3'</td>
<td>7.61 (dd, J=8.5,2.5 Hz, 1H)</td>
<td>7.59 (dd, J = 9, 2 Hz, 1H)</td>
<td></td>
</tr>
<tr>
<td>4'</td>
<td>7.17 (d, J = 8.5 Hz, 1H)</td>
<td>7.13 (d, J = 9 Hz, 1H)</td>
<td></td>
</tr>
<tr>
<td>7'</td>
<td>8.09 (d, J = 2.5 Hz, 1H)</td>
<td>8.06 (d, J = 2 Hz, 1H)</td>
<td></td>
</tr>
</tbody>
</table>

$^a$ 80 MHz (CDCl$_3$-$d^6$DMSO)
Scheme 16: MS fragmentation of phidolopin (36)

\[
\begin{align*}
\text{m/z 152 (38\%)} & \quad \text{m/z 180 (100\%)} \\
\text{m/z 331 (14\%)}
\end{align*}
\]

Scheme 17: MS fragmentation of desmethylphidolopin (37)

\[
\begin{align*}
\text{m/z 166 (100\%)} & \quad \text{m/z 123 (45\%)} \quad \text{HNCO}
\end{align*}
\]
Table 6: $^1$H NMR data for isolated nitrophenols 38-39

<table>
<thead>
<tr>
<th>H on C$^#$</th>
<th>Chemical shift, $\delta$ ppm</th>
<th>38</th>
<th>39</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>8.09 (d, $J = 2$ Hz, 1H)</td>
<td>8.10 (d, $J = 2$ Hz, 1H)</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>7.58 (dd, $J = 9$, 2 Hz, 1H)</td>
<td>7.60 (dd, $J = 8$, 2 Hz, 1H)</td>
</tr>
<tr>
<td>5</td>
<td></td>
<td>7.18 (d, $J = 9$ Hz, 1H)</td>
<td>7.18 (d, $J = 8$ Hz, 1H)</td>
</tr>
<tr>
<td>6</td>
<td></td>
<td>4.69 (s, 2H)</td>
<td>4.43 (s, 2H)</td>
</tr>
<tr>
<td>7</td>
<td></td>
<td>-</td>
<td>3.44 (s, 3H)</td>
</tr>
<tr>
<td>8</td>
<td></td>
<td>10.54 (brs, 1H)</td>
<td>10.58 (s, 1H)</td>
</tr>
<tr>
<td>$\phi$-OH</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

![Diagram of 38 and 39](image-url)
Figure 9: 80 MHz 1H NMR for 4-hydroxymethyl-2-nitrophenol 38
Figure 10: 80 MHz 1H NMR for 4-methoxymethyl-2-nitrophenol 39
Scheme 18: MS fragmentation of nitrophenol 38

\[
\text{38} \\
\begin{align*}
\text{OH} & \quad \text{m/z 123 (25\%)} \\
\text{m/z 152 (11\%)} \\
\text{NO}_2 & \quad \text{m/z 169 (100\%)}
\end{align*}
\]

Scheme 19: MS fragmentation of nitrophenol 39

\[
\text{39} \\
\begin{align*}
\text{OH} & \quad \text{m/z 152 (100\%)} \\
\text{m/z 136 (21\%)} \\
\text{NO}_2 & \quad \text{m/z 183 (59\%)}
\end{align*}
\]
D. DISCUSSION

The isolation of phidolopin (36), desmethylphidolopin (37) as well as nitrophenols (38) and (39) in some combination from the extracts of five Northeast Pacific bryozoans, which all belong to different genera, raises a question about the origin of these secondary metabolites. Natural products which contain unusual functionalities such as nitro groups normally have very restricted taxonomic distributions. Therefore, it seems unlikely that each member of the diverse group of bryozoans investigated in this study would elaborate this same group of novel secondary metabolites through de novo biosynthesis.

A more probable assumption, that these nitrophenols are obtained by the bryozoans from some sort of dietary or symbiotic microorganism such as a phytoplankter, bacterium or fungus, is quite consistent with the organisms characteristic filter feeding capabilities.

A survey of other examples of purine derivatives and nitro containing compounds from marine organisms serves as a useful background for speculation about the origin of the bryozoan metabolites.

Purine derivatives based on the xanthine nucleus are generally of plant origin. Phidolopin (36) represents only the second example of a xanthine alkaloid isolated from a marine source, the first being caffeine (58), isolated from the Chinese gorgonian Echinogorgia pseudossapo.

Marine organisms have also elaborated a number of other purine derivatives not based on the xanthine nucleus. Among the most common
are the purine ribosides such as doridosine (59). In 1980, Cook et al.\textsuperscript{41} reported the isolation of doridosine (1-methylisoguanosine) from the Australian marine sponge \textit{Tedania digitata}. This compound was previously reported from the digestive gland of the dorid nudibranch \textit{Anisodoris nobilis},\textsuperscript{42} and it was found to possess potent muscle
relaxant, anti-inflammatory and anti-allergic activities. The injection of mice with a dose equivalent to lg/Kg of crude sponge extract gave pronounced muscle relaxation and hypothermia which served as a decisive and reproducible assay for purifying 59.

Other biologically active marine purine ribosides include spongosine (2-methoxyadenosine) (60) isolated from the sponge Tedania-digitata and isoguanosine (61), extracted from the dorid nudibranch Diasulula sandiegensis. It is interesting to note that isoguanosine (61) was only found in D. sandiegensis collected from Monterey, California while specimens from La Jolla, California contained only a series of unrelated chlorinated acetylenes.

Adenosine (62) as well as 2'-deoxyadenosine (63) were reported as the cardioactive constituents of the sponge Dasychalina cyathina. Finally 9-β-D-arabinosyladenine (64) and its 3'-O-acetyl derivative
(65), previously known as potent synthetic antiviral agents, have been isolated as natural products from the Italian gorgonian *Eunicella cavolini*.\(^\text{47}\) Compound 64 was the first antiviral drug used in the treatment of the usually fatal herpes encephalitis.

\[
\begin{align*}
\text{62 } R &= \text{OH} \\
\text{63 } R &= \text{H} \\
\text{64 } R &= \text{H} \\
\text{65 } R &= \text{COCH}_3
\end{align*}
\]

Purine derivatives other than ribosides have also been found from marine sources. Two 9-methyladenine derivatives of diterpenes, ageline A (66) and ageline B (67), possessing moderate ichthyotoxic as well as antimicrobial activity were isolated from the Pacific sponge *Agelas sp.*\(^\text{48}\) Five new 9-methyladenine derivatives of diterpenes have been isolated from the Okinawan sea sponge, *Agelas nakamura*.\(^\text{49}\) Four of these metabolites, agelasine-A (68), agelasine-B (69), agelasine-C (70)
and agelasine-D (71) are based on bicyclic diterpene skeletons. The fifth new compound agelasine-E (72), was reported along with the known compound ageline A (66). These 9-methyladeninium salts inhibit Na\(^+\)/K\(^+\)-transporting ATPase in vitro and also possess antimicrobial and antispasmodic properties.
Hokupurine (73) has been isolated from the nudibranch *Phestilla melanobranchia* as well as the coral *Tubastea coccinea* upon which it feeds. In contrast to the other purine derivatives reported from marine sources, hokupurine (73) has no significant biological activity.
This survey of purine metabolites from marine sources shows that nearly all the compounds isolated display some form of significant biological activity. In addition, beginning with the first purine example stated above, caffeine (58), and moving through the compounds of *T. digitata*, *A. nobilis*, *D. sandiegensis* and *P. melanobranchia*, it can be concluded that in most cases the origin of the compounds is not clear. Caffeine (58), for example, is also a well known terrestrial plant natural product. The isolation of caffeine from the Chinese gorgonian *Echinogorgia pseudosappo* can perhaps be rationalized by the characteristic ability of gorgonians to incorporate plant cells in a symbiotic fashion into their tissues.

The occurrence of doridosine (59) in both a sponge and a nudibranch digestive system indicates that the compound originated in the sponge which was grazed upon by the nudibranch. A case could be made for the sponge obtaining the purine derivative from a symbiotic dietary microorganism. Sponges feed on bacteria and they contain many symbiotic microalgae. Observation of ageline A (66) and hokupurine (73) in more than one organism is further proof of the difficulty encountered in the assignment of origin for these purine metabolites.

Nitro containing metabolites are very rare in nature, especially from marine sources. Fungi have been reported to produce a few such compounds. One of the first reported nitro containing compounds was β-nitropropionic acid (74), isolated in 1951 by Bush et al.\(^2\) from *Asperigillus flavus*. This compound was found to have a very low level of antibiotic activity. Two new oxindole alkaloids, cyclopiamine A (75), and its enantiomer, cyclopiamine B (76) have been isolated from
Penicillium cyclopium.\textsuperscript{53} \textit{P. cyclopium} Westling is a frequently encountered fungus on stored grain and cereal products destined for human or animal consumption.

\begin{align*}
\text{NO}_2 & \quad \text{CO}_2\text{H} \\
\text{74} & \\
\text{H} & \\
\text{H} & \\
\text{H} & \\
\text{75} & \\
\text{H} & \\
\text{H} & \\
\text{H} & \\
\text{N} & \\
\text{76} & \\
\text{OCH}_3 & \\
\text{OCH}_3 & \\
\text{77} & \\
\text{CHO} & \\
\text{78} & \\
\text{OH} & \\
\end{align*}

Thaller \textit{et al.}\textsuperscript{54} in 1972 reported isolation of \textit{p}-nitrobenzaldehyde (77) from the fungus \textit{Lepista diemii} Singer.

The first example of a nitro containing phenol appears to be 3,5-dinitroguaiacol (78) reported by Ohta \textit{et al.}\textsuperscript{55} in 1977 from the red
alga Marginisporam aberrans. To the best of this authors knowledge, the isolation of nitrophenols by Ayer et al.\textsuperscript{29} in 1984 was only the second example from the marine environment. It is interesting to note that the low yield of 3,5-dinitroguaiacol (78), 10 mg from 15 Kg of alga, also suggests the possibility of a microbial origin.

In conclusion, the compounds isolated from the Northeast Pacific bryozoans seem to be of a dietary or symbiotic origin. The dietary origin of bryozoan metabolites has been suggested by Pettit et al.\textsuperscript{56a} in the isolation of the bryostatins from two bryozoans, a tunicate and a sponge. The bryostatins appear to be biogenetically related to a number of dinoflagellate toxins such as the pectenotoxins. Similarly, nitrophenols 38 and 39 have recently been isolated by Northcote,\textsuperscript{56b} from a tunicate, Halocynthia iijaboga, and a sponge, Leucosolenia sp., collected in Barkley Sound, B.C. lending further credence to the hypothesis of dietary or symbiotic origin of these compounds. Conformation of this hypothesis could only come through the isolation of the family of nitrophenols from some micro-organism, whether it be phytoplanktonic, bacterial or fungal in nature.
E. INTRODUCTION TO THE SPONGES

Sponges (Phylum Porifera) are the most primitive of multicellular animals. They contain a relatively simple internal organisation, lacking true tissue and organs. All members of the phylum are sessile and exhibit little detectable movement. Sponges are primarily marine animals, except for around 150 fresh water species. They are found in all seas wherever there are rocks, shells, submerged timbers or coral to provide a suitable substratum for attachment.

Sponges vary greatly in size. Certain calcareous species are about the size of a bean, while others can reach the size of a square meter. Some species are radially symmetrical while the majority exhibit irregular, massive, erect, encrusting or branching growth patterns. The size and shape are generally influenced by the nature of the substratum, availability of space, and velocity and type of water current. The water current brings in oxygen and food and removes waste. Remarkably, the volume of water estimated to pass through a sponge 10 cm in height and 1 cm in diameter is about 27.5 L per day.

Marine sponges feed on extremely small particles. It has been demonstrated that the majority of the matter consumed by sponges is of a size undetectable by an ordinary microscope, while the other food consumed consists chiefly of bacteria, dinoflagellates and other plankton. Sponges reproduce asexually by budding or by a variety of processes which involve formation and release of an aggregate of essential cells from the parent sponge. In marine sponges, these aggregates are
called gemmules. Formation of these gemmules in great numbers takes place in the fall before the parent sponge disintegrates with the onset of winter. These gemmules are able to withstand freezing and drying enabling the species to exist through winter. In the spring, the interior cells of the gemmules emerge ultimately developing into an adult sponge.

The approximately 10,000 species of known sponges can be placed within four classes based on the nature of the skeleton. Class Calcarea, comprises all members known as calcreous sponges, distinguished by spicules composed of calcium carbonate. Sponge spicules, made up of calcium carbonate or silicon dioxide deposits, vary in size and shape and often serve as useful characters in identifying sponges. Spicules are generally labelled by the number of axes or rays they possess by adding the appropriate numerical prefix to the ending -axons (when referring to the number of axes) or -actine (when referring to the number of rays or points). The spicules of calcarea are monaxins or three or four pronged types, usually separate. The colours usually encountered in calcreous sponges vary from greyish white to brilliant yellow, red or lavender. Species of this class are the smallest of all sponges (less than 10 cm in height). They can be found in all the oceans of the world, but are generally restricted to shallow waters.

Class Hexactinellida, commonly known as "glass sponges", get their name from the fact that the spicules are always of the triaxon or six pointed type. Also, some of the spicules are occasionally fused to form a lattice like skeleton built of long siliceous fibers, hence their common name. This class elaborates the most symmetrical sponges, which
have cup, vase or urnlike shapes averaging 10 to 30 cm in height. In contrast to Calcarea, Hexactinellidae are mainly deep water sponges, found at depths of 400 to 950 meters mainly in tropical waters of the West Indies and the Eastern Pacific from Japan to the East Indies.

Class Demospongiae contains the greatest number of species including most of the North American sponges. The majority are marine and distributed from shallow water to great depths. Different species are characterized by different bright colours due to pigment granules in their cells. The skeletons vary, consisting of siliceous spicules or spongian fibers or a combination of both. Spicules containing species differ from those in Class Hexactinellida in that their spicules are larger manoxins or tetraxons rather than triaxons.

Finally, Class Sclerospongiae sponges differ from other sponges in having an internal skeleton of siliceous spicules and spongian fibers and an outer encasement of calcium carbonate.

Sponges, particularly those without spicules, often produce large quantities of interesting and biologically active secondary metabolites that are thought to deter potential predators and inhibit growth of fouling organisms. Since many sponges contain symbiotic microorganisms, the true origin of these compounds is at times in question, however, the majority have been attributed to the sponges. As evidence for the use of these compounds for self defence, it has been observed that in the sponge Aplysina fistularis secondary metabolites can only be found in the cells localized adjacent to the exhalent canals. Pioneering studies by Bergmann on the chemical and ecological aspects of marine sponges in the early 1960's initiated intense studies.
leading to the isolation of hundreds of structurally varied natural products especially sterols, many of which were common to terrestrial sources plus a few unique to the marine environment. Due to a lack of sensitive analytical techniques in this period, investigators were limited to the study of only the major components, therefore neglecting the often more interesting trace and minor metabolites.

Studies dating back to the 1940's and 1960's, therefore, yielded mainly "conventional" sterols, that is, sterols which possess the normal 19 carbon nucleus plus an 8 to 10 carbon side chain. Among the most common sterols found in sponges at this time were clionasterol (79) and poriferasterol (80) isolated originally from Cliona celata. Another 29 carbon sterol, chondrillosterol (81), reported as a major component
of *Chondrilla nucula*,\(^{60}\) seemed at the time to be solely confined to sponges.

Among the most widely distributed 27 and 28 carbon sterols in marine organisms are 24-methylenecholesterol (82), found originally in *Chalina arbuscula*,\(^{61}\) cholesterol (83) and cholestanol (84) found as major components in numerous sponges.

![Chemical structural formulas](attachment:image_url)

With the advent of increased analytical technology in the early 1970's the reinvestigation of marine sponges has resulted in the isolation and documentation of sterols possessing completely unprecedented side chain alkylation patterns and modified tetracyclic nuclei. In the
1970's to 1980's reports of unusual sterols began to appear in the literature. Remarkable new side chains including those containing cyclopropane and cyclopropene units, in addition to extensively alkylated side chains were reported.

In 1975, Fattorusso et al.\textsuperscript{62} reported isolation of calysterol (85) from the sponge, \textit{Calyx niceaensis}. Calysterol (85) possessed a cyclopropenyl unit in the side chain, a feature previously observed in nature only in some fatty acids.

\begin{center}
\includegraphics[width=0.5\textwidth]{85.png}
\end{center}

Li et al.\textsuperscript{63} later reported another two steroidal cyclopropenes, (23R),23H-isocalysterol (86) and (24S), 24H-isocalysterol (87) from \textit{Calyx niceaensis}, along with two steroidal cyclopropane derivatives, (23R,24R,28S),23,24-dihydrocalysterol (88) and (23S,24S,28R)-23,24-dihydrocalysterol (89). A novel cyclopropane containing sterol, nicasterol (90), was reported in 1985 by Proudfoot et al.\textsuperscript{64} from \textit{Calyx niceaensis}. The proposed structure was confirmed by partial synthesis.

Many sterols with "extended" side chains have been encountered in sponges. These include aplysterol (91),\textsuperscript{65} stelliferasterol (92) and
isostelliferasterol (93), which all are characterized by the unusual addition of an extra carbon at C-26.

Three further examples of extended side chains are found in verongulasterol (94), a minor sterol from the sponge *Verongia cauliformis*, and in xestosterol (95), a major component, and xestostanol (96), a
minor component of *Xestospongia muta*.\(^{67}\) These three sterols possess the unique feature of being alkylated at both the C-26 and C-27 positions.

Recent isotope feeding studies have led to the demonstration of de novo biosynthesis of xestosterol (95) from desmosterol (97), via a nonstereoselective SAM biomethylation and a 1,2 hydrogen shift (Scheme 20).\(^{68}\)

Modifications to the sterol nucleus are also well documented from sponges. A mixture of stanols having a 19-norcholestanol nucleus carrying either saturated or unsaturated 8, 9 or 10 carbon side chains has been isolated from the sponge *Axinella polypoides*.\(^{69}\) The major component, fully characterized by spectral data as 19-nor-5α,10β-ergost-trans-22-en-3β-ol (98), has been found along with related structures (99)-(105).
Scheme 20: Biosynthesis of xestosterol (95)

- H·

1,2 Shift

101 R = Et

102 R = H, Δ²²-trans

98 R = Me, Δ²²-trans

99 R = H

103 R = Me, Δ²⁴(28)

104 R = Et, Δ²²-trans

105
Examination of the sponge *Axinella verrucosa*\textsuperscript{70} in 1974 led to the isolation of the first example of an A-nor cholestane nucleus from the marine environment. As many as six 3β-hydroxymethyl-A-nor-5α-cholestane sterols, (106)-(111), containing various side chains, were isolated.

\[ \text{HOH}_2\text{C} \quad \text{H} \]

\[ \text{106} \quad R = \text{H} \]
\[ \text{107} \quad R = \text{Me} \]
\[ \text{108} \quad R = \text{Et} \]
\[ \text{109} \quad R = \text{H}, \Delta^{22} \]
\[ \text{110} \quad R = \text{Me}, \Delta^{22} \]
\[ \text{111} \quad R = \text{Et}, \Delta^{22} \]

In 1982, Eggersdorfer et al.\textsuperscript{71} reported the isolation of 3β-hydroxymethyl-A-nor-5α-cholest-15-ene (112) from the pacific sponge,

\[ \text{HOH}_2\text{C} \quad \text{H} \]

\[ \text{112} \]
Homaxinella trachys. This represents the first naturally occurring sterol with a C-15, C-16 double bond.

Biosynthetic tracer incorporation studies carried out by Bibolino et al.\textsuperscript{72} suggest the A-nor sterols can be formed de novo from readily available cholesterol via an enzyme catalyzed ring contraction mechanism involving the loss of the 3\(\alpha\) and 4\(\beta\) hydrogens and the formation of a C-C linkage between C-2 and C-4 (Scheme 21).

\textbf{Scheme 21:} Biosynthetic conversion of cholesterol into 3\(\beta\)-hydroxy-methyl-A-nor-cholestanone
This is the most convincing evidence so far for the ability of sponges to chemically modify sterols subsequent to dietary intake. It has also been found that sponges which contain these A-nor sterols lack sterols with conventional nuclei. This possibly indicates the efficiency of the enzymic system in converting absorbed dietary sterols into A-nor sterols. Therefore, the existence of as yet unknown sterols in nature with conventional nuclei but unusual side chains can be detected in some cases through isolation of the corresponding A-nor sterol.

The most common theories on the diversity of marine sterols appear to be due to the accumulation of several phenomena, including genetic control, biosynthesis by zooxanthellae, commensalism and parasitism, and transmission of molecules by the food chain or by sea water. From a biochemical standpoint, structural variation in the form of extended side chains via biological methylations has been shown to modulate cell plasticity, membrane permeability and ion exchange. The effect of nuclear modification of sterols is as yet unclear, however, it is believed to also play a specialized role in membrane function.
1. Introduction

*Anthoarcuata graceae* (Bakus 1966) is a reddish orange sponge (family Plocamiidae, order Poccilosclida, class Demospongiae, subclass Homoscleromorpha) commonly found in rocky intertidal and subtidal habitats of the B.C. coast. The dorid nudibranch *Aldisa sanguinea cooperi* is often found in close association with the sponge. Most often the nudibranch is deeply embedded in the sponge *A. graceae* from which it obtains nutrition and pigments giving it cryptic coloration.

Our chemical studies on *A. graceae* were prompted by a prior investigation of the chemistry of the nudibranch *A. cooperi* which yielded a mixture of biologically active steroids 113 and 114, a glycerol ether 115 and a complex mixture of minor steroidal ketones, fats and sterols which were not studied further.

*A. graceae*, collected in the shallow waters of Barkley Sound, B.C. at various times of the year was found to be lacking in the major metabolites found in *A. cooperi*, however, a preliminary investigation did reveal the presence of several other minor but very interesting steroids.
2. Isolation and Structure Elucidation

*Anthoarcuata graceae* was collected by hand using SCUBA (1-5 m depth) and immediately immersed in methanol (or ethanol). After extraction for one to three days at room temperature, the methanol (or ethanol) was decanted, vacuum filtered and evaporated in vacuo to yield an aqueous methanolic suspension. This suspension was partitioned between brine and chloroform, and the organic layer was dried over anhydrous \( \text{Na}_2\text{SO}_4 \). The sponge was further soaked in dichloromethane at room temperature for one day, before being ground (with dichloromethane) in a Waring blender. The suspension of ground sponge in dichloromethane was vacuum filtered through celite to yield a filtrate which was stored
at below room temperature. The resulting sponge solids were extracted with dichloromethane a further three times at one day intervals. The combined filtrates were evaporated in vacuo, partitioned between brine and chloroform, and the organic layer was dried over anhydrous Na$_2$SO$_4$. Filtration and evaporation in vacuo of the combined organic layers afforded a dark red crude oil which was fractionated by flash chromatography to give complex mixtures of fats, steroids and pigments detected by analytical TLC analysis. Further purification of the steroidal fraction by column chromatography (Sephadex LH-20) and reverse phase HPLC yielded two steroidal fractions as white solids. Analysis of these two fractions by $^1$H and $^{13}$C NMR provided evidence for the presence of a mixture of components in each. Further separation and purification of each mixture was carried out by preparative TLC yielding two novel $\Delta^4$-3,6-diketosteroids 116 and 117, two unprecedented A-nor-steroids anthosterone A (118) and anthosterone B (119) as well as two diosphenols tentatively assigned structures 120 and 121.

3A. $\Delta^4$-3,6-diketosteroids

Compound A, mp 92-93°C, had a molecular formula of C$_{27}$H$_{40}$O$_2$ (HRMS 396.3034, calcd. 396.3030) that required 8 units of unsaturation. The $^1$H NMR indicated methyl resonances at $\delta$ 0.73 (s, 3H), 0.95 (d, J = 6 Hz, 3H), and 1.17 ppm (s, 3H), corresponding to the C-18, C-21, and C-19 methyls of a conventional steroid. In addition, two olefinic methyl $^1$H NMR resonances were found at $\delta$ 1.61 (s, 3H), 1.69 (s, 3H), as well as an
olefinic triplet at 5.09 (t, J = 6.4 Hz, 1H), ppm, which indicated the presence of a Δ24,25 double bond in the side chain. This was further supported by an IR absorbance at 1643 cm⁻¹ and ¹³C NMR resonances at δ 124.99 (d), 127.09 (s) ppm which could be assigned to the C-24 and C-25 carbons of the side chain.

One remaining olefinic signal in the ¹H NMR at δ 6.19 (s, 1H) ppm (Figure 11), in addition to four downfield resonances in the ¹³C NMR, indicated the presence of some unique functionality in the A and B rings. The ¹³C NMR resonances at δ 202.36 (s) and 199.56 (s) ppm were reminiscent of the carbonyl carbon resonances found in Δ⁴-3-ketosteroids (Table 8), while the remaining two resonances at δ 125.48 (d) and 161.06 (s) ppm could be assigned to the corresponding Δ⁴-double bond. Infrared bands at 1606 (νc = c) and 1686 (νc = o)cm⁻¹, coupled with a UV λ_max at 248.9 nm (ε 11600) representing an enone with extended conjugation, allowed the tentative assignment of compound A 116 as an ene-dione.

Confirmation of the assigned structure was achieved by the synthesis of a model Δ⁴-3,6-diketosteroid system (Scheme 22). This was

Scheme 22: Jones oxidation of cholesterol (83)
Figure 11: 400 MHz $^1$H NMR of compound 116
Figure 12: 400 MHz $^1$H NMR of compound 122
Figure 13: 75 MHz $^{13}$C NMR of compound 122
Table 7: $^1$H NMR Data for $\Delta^4$-3,6-Ketosteroids (CDCl$_3$)

<table>
<thead>
<tr>
<th>H on C#</th>
<th>116$^a$</th>
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<th>122$^a$</th>
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<td>.73 (s, 3H)</td>
<td>.74 (s, 3H)</td>
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<td>1.17 (s, 3H)</td>
<td>1.19 (s, 3H)</td>
<td></td>
</tr>
<tr>
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<td>.94 (d, J=6 Hz, 3H)</td>
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</tr>
<tr>
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<td>5.09 (t, J=6.4 Hz, 1H)</td>
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<td>-</td>
<td></td>
</tr>
<tr>
<td>26</td>
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<td>.87 (d, J=6 Hz, 3H)</td>
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<tr>
<td>27</td>
<td>1.61 (s, 3H)$^b$</td>
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<td>4.65/4.73</td>
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$^a$ 400 MHz
$^b$ shifts interchangeable
Figure 14: 400 MHz $^1$H NMR of compound 117
Figure 15: 75 MHz $^{13}$C NMR of compound 117
Table 8: $^{13}$C NMR Data for $\Delta^4$-3,6-diketosteroids (CDCl$_3$)

<table>
<thead>
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<th>(122)</th>
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<td>199.40 (s)</td>
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<td>125.48 (d)</td>
<td>125.44 (d)</td>
<td>125.33 (d)</td>
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<td>5</td>
<td>161.06 (s)</td>
<td>161.08 (s)</td>
<td>160.98 (s)</td>
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<td>6</td>
<td>202.36 (s)</td>
<td>202.36 (s)</td>
<td>202.25 (s)</td>
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<tr>
<td>24</td>
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<td>127.09 (s)</td>
<td>-</td>
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</tr>
<tr>
<td>28</td>
<td>-</td>
<td>106.06 (t)</td>
<td>-</td>
</tr>
</tbody>
</table>
Scheme 23: MS fragmentation of compound B 117

m/z 410 → m/z 326 (13 %)

-C₆H₁₂

m/z 137 (80 %)
achieved via a Jones oxidation\textsuperscript{76} of cholesterol (83) which after purification by preparative TLC gave $\Delta^4$-cholestane-3,6-dione (122) as one of the major products. The HRMS of 122 gave a parent ion corresponding to a molecular formula of C$_{27}$H$_{42}$O$_2$ (HRMS 398.3186, calcd. 398.3187). A $^1$H NMR spectrum of 122 (Table 7) showed a sharp olefinic signal at $\delta$ 6.19 (s, 1H) ppm (Figure 12) and its $^{13}$C NMR spectrum (Figure 13) had resonances at $\delta$ 199.40 (s) and 202.25 (s) corresponding to the carbonyl carbons (C-3, C-6) plus two olefinic resonances at $\delta$ 125.33 (d) and 160.98 (s) ppm (C-4, C-5) assigned to the $\Delta^4,5$ double bond system (Table 7). The IR spectrum of 122 showed absorbances at 1685 and 1606 cm$^{-1}$ in close agreement to the natural product.

Co-occurring with Compound A 116 was a related steroid, Compound B 117, mp 111-112°C, which possessed a $\Delta^{24,28}$ double bond in the side chain. Compound B 117 had a molecular formula C$_{28}$H$_{42}$O$_2$ (HRMS 410.3190, calcd. 410.3187) differing from compound A 116 by 14 mass units (=CH$_2$). The $^1$H NMR (Figure 14) showed methyl resonances at $\delta$ 0.73 (s, 3H), 0.85 (d, $J$ = 7 Hz, 3H), 1.02 (d, $J$ = 8 Hz, 6H), 1.17 (s, 3H) ppm corresponding to the C-18, C-21, C-26/27 and C-19 methyls respectively, of the steroidal skeleton (Table 7). $^1$H NMR olefinic resonances at $\delta$ 4.65 (s, 1H) and 4.73 (s, 1H) ppm, in addition to $^{13}$C NMR (Figure 15) signals at $\delta$ 106.06 (t) and 156.65 (s) ppm, are typical of an olefinic-methylene at the C-24/28 position (Table 8). This is further supported by the strong peak at m/z 326 in the mass spectrum indicating a loss of the C$_6$H$_{12}$ side chain via a McLafferty rearrangement (Scheme 23). Identical functionality in the A and B rings was confirmed by the presence of a sharp olefinic singlet at $\delta$ 6.17 (s, 1H) ppm in the $^1$H NMR, and by
resonances at δ 125.44 (d), 161.08 (s) (Δ⁴,⁵-double bond), 199.56 (s), 202.36 (s) ppm (carbonyl carbons) in the ¹³C NMR. Compounds A and B had comparable UV and IR spectral data.

3B. A-Nor Steroids

Examination of the more polar band from the preparative TLC purification yielded two related compounds anthosterone A (118) and anthosterone B (119). Anthosterone A (118), mp 142-143°C, had a molecular formula C₂₈H₄₂O₄ (HRMS 442.3083, calcd. 442.3079) requiring 8 units of unsaturation. Its ¹H NMR spectrum gave methyl signals at δ 0.73 (s, 3H), 0.96 (d, J = 6.7 Hz, 3H), and 1.21 (s, 3H) ppm corresponding to the C-18, C-21 and C-19 methyls of a steroid skeleton, in addition to two signals assigned to olefinic methyl groups (C-26/C27) at δ 1.62 (s, 3H), and 1.69 (s, 3H) ppm. Further examination of the ¹H NMR spectrum (Figure 16) indicated the presence of an olefinic triplet at δ 5.10 (t, J = 8 Hz, 1H) ppm which could be assigned to a proton on C-24 of a Δ²⁴,²⁵ double bond in the side chain (Table 9). ¹³C NMR olefinic resonances at 125.09 (d) and 131.05 (s) supported this assignment (Table 10). Additional ¹H NMR resonances consisted of an AB quartet made up of doublets resonating at δ 2.08 (d, J = 13.3 Hz, 1H) and 2.19 (d, J = 13.3 Hz, 1H), one sharp methyl singlet at 3.79 (s, 3H), and a deshielded olefinic proton at 6.73 (t, J = 3.3 Hz, 1H) ppm.
Figure 18: 400 MHz 1H NMR of anthosterone B 119
Table 9: $^1$H NMR data for compounds 118 and 119

<table>
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<th>119$^a$</th>
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</thead>
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<td>6.73 (t, J=2.5 Hz, 1H)</td>
</tr>
<tr>
<td>18</td>
<td>0.73 (s, 3H)</td>
<td>0.73 (s, 3H)</td>
</tr>
<tr>
<td>19</td>
<td>1.21 (s, 3H)</td>
<td>1.20 (s, 3H)</td>
</tr>
<tr>
<td>21</td>
<td>0.96 (d, J=6.7 Hz, 3H)</td>
<td>0.97 (d, J=6.5 Hz, 3H)</td>
</tr>
<tr>
<td>24</td>
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<td>29</td>
<td>-</td>
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</table>

$^a$ 400 MHz, CDCl$_3$

$^b$ shifts interchangeable

![Chemical structures of 118 and 119](image-url)
Table 10: $^{13}$C NMR data for compounds 118 and 119

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<td>22.01 (t)</td>
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<td>153.53 (s)</td>
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<td>33.81 (s)</td>
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<td>53.40 (q)</td>
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</table>

† Assignment based on APT experiment (Figure 20).

a 75 MHz, CDC13

b Assignments interchangeable
c Assignment interchangeable
Assignment of the sharp methyl singlet at δ 3.79 was aided by the observation of a MS fragment at m/z 383.2932 (calcd. 383.2952) indicating an $M^+\text{-59}$ loss corresponding to C$_2$H$_3$O$_2$ of a methyl ester functionality. The presence of the methyl ester moiety was further confirmed by $^{13}$C NMR resonances at δ 173.37 (s) (C-4, C-0) and 55.91 (q) (C-29, CH$_3$) ppm (Figure 17) along with an IR band at 1744 cm$^{-1}$.

An interesting absorbance in the IR spectrum at 1717 cm$^{-1}$, coupled with a $^{13}$C NMR carbonyl signal at δ 200.91 (s), two olefinic signals at δ 136.28 (d) and 145.10 (s) ppm, and a UV $\lambda_{\text{max}}$ at 250.2 (ε 12500) nm suggested the presence of an enone system as seen in compounds A and B.

The mass spectrum of 118 also showed a strong peak at m/z 330.1810 (C$_{20}$H$_{26}$O$_4$, calcd. 330.1832) indicating a $M^+\text{-113}$ (C$_8$H$_{17}$) loss corresponding to the cleavage of the side chain (Scheme 24). With the further loss of 60 daltons (C$_2$H$_4$O$_2$) as indicated by a peak at m/z 270.1597 (calcd. 270.1621), the remaining nucleus after the loss of the methyl ester yielded a molecular formula C$_{18}$H$_{22}$O$_2$ which first indicated the presence of a nor-sterol (Scheme 24). With all the methyl groups accounted for, a logical conclusion to draw was the presence of an A-nor ring.

Co-occurring with anthosterone A (118) was the related steroid, anthosterone B (119), mp 155-157°C, which had a molecular formula C$_{29}$H$_{44}$O$_4$ (HRMS 456.3236, calcd. 456.3241) requiring 8 units of unsaturation. The $^1$H NMR (Figure 18) showed methyl signals at δ 0.73 (s, 3H), 0.97 (d, $J = 6.5$ Hz, 3H), 1.04 (d, $J = 6.5$ Hz, 6H), 1.20 (s, 3H) ppm assigned to the C-18, C-21, C-26/27 and C-19 methyls respectively. A difference in molecular weight of 14 daltons (CH$_2$) plus olefinic
Scheme 24: MS fragmentation of anthosterone A (118)

![Scheme 24: MS fragmentation of anthosterone A (118)](image)

resonances in the $^1$H NMR at $\delta$ 4.65 (s, 1H), and 4.71 (s, 1H) ppm indicated the presence of a C-24 (28) olefinic-methylene system (Table 9). This assignment was supported by the $^{13}$C NMR resonances at 106.01 (t) and 156.79 (s) ppm (Figure 19) which are diagnostic for this type of system. The remaining portions of the $^1$H NMR spectrum including the presence of the AB quartet (2.10/2.18, d, $J = 13.3$ Hz, 2H), a sharp singlet at $\delta$ 3.77 (s, 3H) for the methyl ester as well as an olefinic triplet at $\delta$ 6.73 (t, $J = 3$ Hz, 1H) ppm indicated that the basic nucleus
of anthosterones A and B was identical. This was supported by the $^{13}$C NMR spectrum which showed a deshielded carbonyl resonance at $\delta$ 200.91 (s), two olefinic resonances at 136.29 (d) and 146.08 (s) ppm (Table 10) and the mass spectrum which also showed a strong peak at 298.1928 (calcd. 298.1934) corresponding to the C$_{20}$H$_{26}$O$_2$ nucleus (Scheme 25).

Scheme 25: MS fragmentation of anthosterone B (119)

![Scheme 25](image)

Since the difference between the two compounds was only in their side chains, the remaining objective was to propose a structure for the common nucleus.

Further examination of the $^{13}$C NMR spectrum of anthosterone A (118) showed the presence of a carbon singlet at $\delta$ 79.90 (s) assigned to a
tertiary carbinol carbon. The presence of a hydroxyl group was supported by the mass spectral loss of HOH (Scheme 24). As expected then, anthosterone B (119) could be acetylated with acetic anhydride/pyridine at room temperature for 12 hr to afford anthosterone B-acetate (123) 498.3336 (calcd. 498.3347) in quantitative yield (Scheme 26).

Scheme 26: Acetylation of anthosterone B (119)

The 1H NMR spectrum of 123 (Figure 21) showed an upfield shift in the C-19 methyl resonance from δ 1.20 in 119 to 1.10 ppm in 123, as well as a downfield shift in both protons making up the AB system, with the doublets at δ 2.10 and 2.18 in 119 shifting to 2.47 and 2.65 ppm respectively in 123 (Table 11). This indicated a close proximity in the
Figure 21: 270 MHz 1H NMR of anthosterone B-acetate 123
Table 11: $^1$H NMR comparison between 119 and 123

![Chemical structure](image)

<table>
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<tr>
<th>H on C#</th>
<th>Chemical shift, $\delta$ ppm</th>
<th>$^{119}_a$</th>
<th>$^{123}_a$</th>
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<tr>
<td></td>
<td>2.18 (d, J=13.5 Hz, 1H)</td>
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<tr>
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<tr>
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<td>1.20 (s, 3H)</td>
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<td>4.65/4.71 (s, 1H)</td>
<td>4.67-4.73 (s, 1H)</td>
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</table>

$^a$
nucleus between the hydroxyl group, the C-19 methyl and the AB spin system. A difference NOE experiment, in which the C-19 methyl singlet of anthosterone B (119) was irradiated, showed an enhancement of the upfield portion of the AB quartet showing the close spacial proximity of these functionalities. Additional NOE experiments included irradiation of the olefinic triplet at δ 6.73 ppm which induced an enhancement of allylic proton multiplets at δ 1.88 (m, 1H), and 2.41 (m, 1H) ppm and irradiation of the multiplet at δ 2.41 (m, 1H) yielding an NOE enhancement of the triplet at δ 6.73 as well as its apparent geminal partner at 1.88 (m, 1H) ppm. Irradiation of the olefinic signal at δ 6.73 in a double resonance experiment resulted in sharpening of signals at 1.88 and 2.40 ppm, while irradiation of the multiplet at δ 2.40 changed the triplet at 6.73 to a doublet and sharpened the signal at 1.88 ppm. The 13C NMR spectrum showed two olefinic signals, δ 136.28 (d) and 145.10 (s) ppm, which could be assigned to a tri-substituted alkene with an adjacent allylic methylene (Figure 22).

Figure 22: Allylic methylene system
The next major obstacle was the positioning of the ketone carbonyl which from the UV data had to be conjugated to a double bond. Assuming the presence of the contracted A ring, it was possible to propose structures based on all the accumulated data which possessed either a 4-keto or a 6-keto functionality. Three possible structures A, B, and C were proposed which accommodated all the functionality required by the data, including the methyl ester, enone system, tertiary alcohol as well as the five membered A ring.

\[ \text{CH}_3\text{O} - \text{CO} \]

A SINEPT\(^7^8\) (selected insensitive nuclei enhanced by polarization transfer) experiment eliminated one of the possibilities based on observed polarization transfer through 2 and 3 bond \( ^1\text{H} - ^{13}\text{C} \) couplings. A soft pulse applied to the upfield proton of the AB system (\( \delta \ 2.18 \text{ ppm} \)) afforded enhancements of \( ^{13}\text{C} \) NMR signals at 200.91 (s), 173.36 (s),
145.08 (s) and 79.90 (s) ppm indicating $^1\text{H} - ^{13}\text{C}$ coupling to the $\alpha,\beta$ unsaturated ketone carbonyl, the methyl ester carbonyl, the non-protonated olefinic and the carbinol carbons (Figure 23). Only structures A and C could fit this data while with structure B, a required 6-bond coupling to the methyl ester carbonyl carbon would not be expected.

A two dimensional homonuclear COSY$^{79}$ experiment confirmed that the AB system was not spin isolated as indicated by an observed coupling between the downfield member of the AB system at $\delta$ 2.18 and the C-19 methyl singlet at 1.20 ppm (Figure 24). This observed coupling (W-coupling) between the methyl group and the downfield $\alpha$-proton provided strong evidence for the proposed structure C. The relative stereochemistry at the C-2 center with the hydroxyl group in the $\beta$ orientation, was assigned based on the observed upfield shift of the C-19 methyl resonance (1.20 to 1.10 ppm) on acetylation of anthosterone B (119).

Confirmation of the structure was provided by a single crystal X-ray diffraction analysis$^{80}$ on anthosterone A (118). Figure 25 shows a computer generated ORTEP drawing of 118.
Figure 23: SINEPT experiment on anthosterone B 119
Figure 24: 2D Homonuclear COSY on anthosterone B (119)

Figure 25: Computer generated ORTEP drawing of anthosterone A 118
3C. Diosphenols 120 and 121

An investigation of two steroids which were more polar than the A-nor sterols on preparative TLC, and present in trace amounts, has led to a preliminary structural proposal based on spectral data. Compound E 120, had a molecular formula C_{27}H_{42}O_{3} (HRMS 414.3149, calcd 414.3136) requiring 7 units of unsaturation. The ^{1}H NMR spectrum gave methyl signals at δ 0.73 (s, 3H), 0.93 (d, J = 6 Hz, 3H), and 1.28 (s, 3H) ppm assigned to the C-18, C-21 and C-19 methyls of a steroid nucleus. Two olefinic methyl groups (C26/27) at δ 1.52 (s, 3H) and 1.69 (s, 3H) plus an olefinic triplet at 5.09 (t, J = 7 Hz, 1H) ppm indicated the presence of a trisubstituted side chain double bond as seen in compound A 116 and anthosterone A (118). Further examination of the ^{1}H NMR (Figure 26) revealed an AB system, δ 2.18 (d, J = 16 Hz, 1H) and 2.25 (d, J = 16 Hz, 1H), a sharp singlet at 3.29 (s, 3H), a deshielded methine proton at
Figure 26: 400 MHz $^1$H NMR of compound E 120
4.52 (t, J = 3 Hz, 1H) as well as an olefinic singlet at 5.28 (s, 1H) ppm (Table 12). Based on a loss in the mass spectrum of 32 daltons (MeOH), the three proton singlet at 3.29 was assigned to a methyl ether functionality (Scheme 27).

**Scheme 27: MS fragmentation of 120**

![Scheme 27: MS fragmentation of 120](image)

Infrared bands at 3438 (νO-H), 1711, 1674 (νC=O) and 1606 (νC=C) cm⁻¹, plus a UV absorbance 264 nm (ε 10971) which shifted to 304 nm (ε 9731) on addition of base, strongly indicated the presence of a diosphenol functionality based on a comparison with literature values. The presence of the diosphenol was further supported by the positive TLC spray test with 2,4-dinitrophenylhydrazine and FeCl₃.
Table 12: $^1$H NMR data on diosphenols 120 and 121

<table>
<thead>
<tr>
<th>H on C#</th>
<th>Chemical shift, δ ppm</th>
<th>120$^a$</th>
<th>121$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.18/2.25 (d, J=16 Hz, 1H)</td>
<td>2.17/2.29 (d, J=16 Hz, 1H)</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>5.28 (s, 1H)</td>
<td>5.28 (s, 1H)</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>4.52 (t, J=3 Hz, 1H)</td>
<td>4.57 (t, J=2 Hz, 1H)</td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>0.73 (s, 3H)</td>
<td>0.74 (s, 3H)</td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>1.28 (s, 3H)</td>
<td>1.31 (s, 3H)</td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>0.93 (d, J = 6 Hz, 3H)</td>
<td>0.94 (d, J = 8 Hz, 3H)</td>
<td></td>
</tr>
<tr>
<td>23</td>
<td>5.09 (s, 1H)</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>1.52$^b$ (s, 3H)</td>
<td>1.03 (d, J = 7 Hz, 6H)</td>
<td></td>
</tr>
<tr>
<td>26</td>
<td>1.69$^b$ (s, 3H)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>27</td>
<td>3.29</td>
<td>4.76 (s, 1H)</td>
<td></td>
</tr>
<tr>
<td>28</td>
<td>-</td>
<td>3.33 (s, 3H)</td>
<td></td>
</tr>
</tbody>
</table>

$^a$ 400 MHz, CDCl$_3$

$^b$ shifts interchangeable
Irradiation of the C-19 methyl singlet at δ 1.28 in a difference NOE experiment on compound E 120, resulted in an enhancement of the methoxy singlet at 3.29 and the upfield component of the AB system at 2.18 (d, J = 16 Hz, 1H) ppm. Irradiation of the triplet at δ 4.52 ppm afforded only an enhancement of the methoxy singlet. Decoupling of the triplet at δ 4.52 sharpened allylic signals at 2.10 as well as proton signals in the aliphatic region at 1.00 to 1.20 ppm. A two dimensional homonuclear COSY experiment on compound E further suggested coupling between the proton at δ 4.52 and an allylic proton at 2.10 and aliphatic protons at 1.00 to 1.20 ppm (Figure 27).

Co-occurring with compound E 120 was the related compound F 121, which had a molecular formula of C$_{28}$H$_{44}$O$_3$ (HRMS 428.3284, calcd. 428.3291). A $^1$H NMR of compound F 121 afforded signals at δ 0.74 (s, 3H), 0.94 (d, J = 7 Hz, 3H), 1.03 (d, J = 6.4 Hz, 3H), and 1.31 (s, 3H) ppm assigned to the C-18, C-21, C-26/27 and C-19 methyls respectively. The presence in the $^1$H NMR (Figure 28) of sharp olefinic singlets at δ 4.69 (s, 1H) and 4.76 (s, 1H) ppm (Table 12) plus an additional 14 mass units (=CH$_2$) in the mass spectrum indicated the presence of an olefinic-methylene functionality in the side chain as seen in compounds 117 and 119.

Subtracting the two carbons assigned to the methyl ether and olefinic methylene from the total of 28 carbons in the molecular formula of compound F, leaves a total of only 26 carbon atoms. Therefore compound F cannot have a standard steroidal skeleton. Further examination of the mass spectrum of compound F 121 revealed a strong peak at m/z 344.2351 (calcd. 344.2353) indicating a loss of 84 daltons (C$_6$H$_{12}$)
Figure 28: 400 MHz $^1$H NMR of compound F 121
assigned to loss of a truncated side chain via a McLafferty rearrangement (Scheme 28). A similar fragmentation pattern was observed for compound E.

Scheme 28: MS fragmentation of compound F 121

This evidence allowed the assignment of shortened side chains in both compounds E and F.

Based on the spectroscopic data accumulated in these two compounds, tentative structures 120 and 121 were assigned pending further isolation of sufficient material which would facilitate an unambiguous assignment of their structures.
3D. Biosynthesis of Sponge Metabolites

A biosynthetic proposal for the formation of the new steroids isolated from Anthoarcuata graceae could logically begin with Δ²⁴-cholesterol. A proposed biogenetic pathway for the two Δ⁴-3,6-diketosteroids 116 and 117 is outlined in Scheme 29. The side chain found in compounds 117 and 119 could be formed by biomethylation with S-adenosylmethionine (SAM) and allylic rearrangement (steps 2 and 3). Allylic rearrangement of the double bond (step 4) followed by oxidation of the 3β-hydroxyl group (step 5) and allylic hydroxylation and oxidation at the C-6 position (steps 6, 7) represents a possible biogenetic route to compounds 116 and 117.

A mixture of steroids including 4,7,22-triene-3,6-diketones 124 have been isolated by Malorni et al.⁸⁴ from the marine sponge, Raphidostila incisa, collected off Zlarin, Yugoslavia, and cholesta-4,7-diene-3,6-dione (125), was isolated by Kinnear et al.⁸⁵ from the insect,
Scheme 29: Biosynthetic proposal for compounds A and B
Calliphora stygia. It appears, however, that compounds 116 and 117 are the only examples of naturally occurring Δ^4-3,6-diketosteroids from marine sources.

A possible biosynthetic pathway to the anthosterones A (118) and B (119) proceeds through a diosphenol intermediate as seen in Scheme 30. If the diosphenol is converted to the α-diketone tautomer (Step 1), contraction of the A ring can occur via a benzylic acid rearrangement (Step 2) to give the α-hydroxy carboxylic acid intermediate, which on hydroxylation and oxidation (Step 3), followed by methylation (Step 4) would form the common nucleus found in natural products 118 and 119. It appears from a literature search that the nucleus found in compounds 118 and 119 is unprecedented. It is not completely clear yet whether these unusual A-nor steroids are metabolites elaborated by the sponge or artifacts formed during isolation. An ethanol extract was examined in an attempt to isolate the ethyl ester derivative. This experiment was inconclusive due to insufficient material.

A proposed pathway to the diosphenols 120 and 121 is outlined in Scheme 31 with the addition of methanol to the C-6 position. Examples of side chains shortened by one carbon are known from marine organisms. A previous biosynthetic proposal involved the methylation of C-24 with SAM, followed by oxidative degradation to lose C26/27 of the side chain (Scheme 32). Subsequent methylations and reductions could yield the side chains proposed for compounds 120 and 121. The side chains proposed for compounds 120 and 121 appear to be the first naturally occurring examples of these side chains.
Scheme 30: Biosynthetic proposal for A-nor steroids 118, 119
Scheme 31: Proposed formation for diosphenols 120, 121

Scheme 32: Biosynthetic proposal for shortened side chains
G. EXPERIMENTAL

The $^1$H NMR spectra were recorded on either the Bruker-WH-400, Bruker WP-80, Nicolet-Oxford 270 or Varian XL-300 spectrometers. Tetramethylsilane ($\delta = 0$) was employed as the internal standard for $^1$H NMR spectra and CDCl$_3$ ($\delta = 77.0$ ppm) or DMSO-d$_6$ ($\delta = 39.5$ ppm) were used both as internal standards as well as solvents for $^{13}$C NMR spectra unless otherwise indicated.

Low resolution and high resolution electron impact mass spectra were recorded on Kratos MS-59 and MS-50 spectrometers respectively. Infrared spectra were recorded on a Perkin-Elmer 1710 FT spectrometer and UV absorption spectra were measured on a Bausch and Lomb Spectronic 2000 spectrometer. Optical rotations were measured on Perkin-Elmer model 141 polarimeter using a 10 cm cell, while uncorrected melting points were determined on a Fisher-Johns melting point apparatus.

HPLC was carried out on either a Perkin-Elmer Series 2 instrument equipped with a Perkin-Elmer LC-55 UV detector or a Waters model 501 system equipped with a Waters 440 dual wavelength detector for peak detection. The HPLC column used was the Whatman Magnum-9 ODS-3 reverse phase preparative column. The solvents used for HPLC were BDH Ombisolve or Fisher HPLC grade and the water used was glass-distilled. All other solvents used were at least reagent grade unless otherwise indicated.

Silica gel types used were Merck silica gel 60 PF-254 for preparative TLC, Merck silica gel 60 230-400 mesh for flash chromatography and Merck silica gel 60 PF-254 with CaSO$_4$ 1/2H$_2$O for radial TLC. All $R_f$
values were calculated on analytical TLC plates using Macherey-Nagel Sil G/UV 254 precoated sheets .25 mm thick.

Preparation of methylated 4-methyl-2-nitrophenol 41

To a stirred suspension of potassium carbonate (1.500 g, 10.9 mmol) in distilled acetone (7 ml) in a 25 ml round bottom flask was added 4-methyl-2-nitrophenol (1.000 g, 6.5 mmol) dissolved in acetone (5 ml). The resulting yellow solution turned completely red in about 2 minutes. After this, methyl iodide (1.800 g, 12.7 mmol) was added dropwise and the reaction mixture was refluxed for approximately 40 minutes while being monitored by TLC (10:90 ethyl acetate/petroleum ether). The red reaction mixture was vacuum filtered and the resulting filtrate was poured into a separatory funnel and partitioned between water (25 ml) and chloroform (4 x 30 ml). The chloroform layers were combined, dried over anhydrous Na₂SO₄, and filtered. Evaporation in vacuo yielded a yellow gum which on purification by preparative TLC (10:90 ethyl acetate/petroleum ether) gave compound 41, 450.6 mg (41%) as a yellow oil: ¹H NMR (80 MHz, CDCl₃) δ 2.35 (s, 3H), 3.95 (s, 3H), 6.99 (d, J = 9 Hz, 1H), 7.35 (dd, J = 9, 2 Hz, 1H), 7.60 (d, J = 2 Hz, 1H) ppm; HRMS, m/z observed 167.0583, required for C₈H₈N0₃ 167.0583; MS m/z (relative intensity) 167 (78), 137 (49), 120 (82), 105 (27), 91 (100), 77 (50), 65 (75).
Preparation of Brominated Compound 51

Purified N-bromosuccinimide (0.155 g, 0.87 mmol) was added to a refluxing solution of 41 (0.124 g, 0.81 mmol) dissolved in carbon tetrachloride (5 ml) in a 10 ml round bottom flask. The refluxing mixture was irradiated for 1 hr with a 150 W tungsten light bulb. After cooling, the mixture was filtered and the filtrate was concentrated in vacuo. The resulting yellow heavy oil was purified by preparative TLC (3:10 ethyl acetate/hexanes) giving a clear yellow oil (Rf. 51), 0.089 g (45%): MS, m/z (relative intensity) 247 (2), 245 (3), 166 (65), 134 (51), 120 (25), 91 (34), 77 (62).

Preparation of methylated phidolopin 53

Purified compound 51 (0.080 g, 0.36 mmol) was dissolved in tetrahydrofuran (3 ml) and added dropwise to a stirring solution of theophylline (0.051 g, 0.28 mmol) in 0.1 M NaOH (2.5 ml). After addition was complete, the reaction mixture was refluxed for 1 hr. At the end of this time the reaction mixture was cooled and partitioned between distilled water (15 ml) and chloroform (4 x 20 ml). The chloroform layer was dried over anhydrous Na$_2$SO$_4$ and filtered. Evaporation of the solvent followed by purification by preparative TLC (ethyl acetate) yielded 0.043 g (81%) of compound 53 as a white solid: $^1$H NMR (80 MHz, CDCl$_3$) 3.43 (s, 3H), 3.58 (s, 3H), 5.48 (s, 2H), 7.00 (d, J = 9 Hz, 1H), 7.38 (dd, J = 9, 2 Hz, 1H), 7.68 (d, J = 2 Hz, 1H) ppm; HRMS, m/z
observed 345.1075, required for C\textsubscript{15}H\textsubscript{15}N\textsubscript{5}O\textsubscript{5} 345.1075; MS, m/z (relative intensity) 345 (M, 7), 180 (23), 166 (100), 91 (21), 77 (14).

Preparation of the MOM protected 4-methyl-2-nitrophenol 42

To a stirred suspension of anhydrous potassium carbonate (0.897 g, 6.5 mmol) in distilled acetone (20 ml) under a nitrogen atmosphere in a 50 mL round bottom flask was added 4-methyl-2-nitrophenol (1.000 g, 6.5 mmol) dissolved in acetone (5 ml). The resulting yellow solution turned deep red after 15-20 minutes at room temperature. Anhydrous chloromethymethylmethyl ether (1.50 ml, 19.5 mmol) was added dropwise and the resultant yellow solution was stirred for 10 minutes. The reaction mixture was filtered and the filtrate was added to a 150 ml separative funnel containing 10 ml distilled water. The aqueous layer was repeatedly extracted with portions of chloroform (25 ml) until the yellow color had disappeared. The combined organic layer was dried over anhydrous Na\textsubscript{2}SO\textsubscript{4} and filtered. Evaporation of the solvent in vacuo followed by preparative TLC (1:10 ethylacetate/hexanes) yielded 0.930 g (73%) of compound 42 as a yellow oil: \textsuperscript{1}H NMR (80 MHz, CDCl\textsubscript{3}) \delta 2.35-3.53 (s, 3H), 5.25 (s, 2H), 7.13 (bd, J = 9 Hz, 1H), 7.30 (dd, J = 9, 2 Hz, 1H), 7.56 (d, J = 2 Hz, 1H) ppm; HRMS, m/s observed 197.0693, required for C\textsubscript{9}H\textsubscript{11}NO\textsubscript{4} 197.0688; MS m/z (relative intensity) 197 (55), 167 (34), 166 (2.5), 136 (11), 45 (100).
Preparation of MOM Phidolopin 55

Purified N-bromosuccinimide (0.242 g, 1.36 mmol) was added to a refluxing solution of 42 (0.0891 g, .45 mmol) dissolved in carbon tetrachloride (5 ml) in a 10 ml round bottom flask. The refluxing mixture was irradiated for 15 minutes with a 250 W sun lamp. After cooling, the mixture was filtered and the filtrate was concentrated in vacuo. The resulting brown solid was dissolved in tetrahydrofuran (5 ml) and added dropwise to a solution of theophylline (0.0823 g, .045 mmol) and .1 M sodium hydroxide (2.5 ml). After addition was complete, the reaction mixture was stirred at room temperature for 36 hours. At the end of this time the reaction mixture was partitioned between distilled water (15 ml) and chloroform (4 x 20 ml). The chloroform layer was dried over anhydrous Na$_2$SO$_4$ and filtered. Evaporation of the solvent followed by purification by preparative TLC (ethyl acetate) gave .534 g (32%) of pure 55 as a white solid: $^1$H NMR (80 MHz, CDCl$_3$) $\delta$ 3.40 (s, 3H), 3.50 (s, 3H), 3.71 (s, 3H), 5.25 (s, 2H), 5.46 (s, 2H), 7.28 (bd, $J$ = 8.5 Hz, 1H), 7.53 (dd, $J$ = 8.5, 2 Hz, 1H), 7.63 (s, 1H), 7.75 (d, $J$ = 2.1 Hz, 1H) ppm; HRMS, m/z observed 375.1178, required for C$_{16}$H$_{17}$N$_5$O$_6$ 375.1180; MS m/z (relative intensity) 375 (8), 393 (M$^+$ + H$_2$O, 2), 330 (6), 313 (21), 258 (27), 209 (32), 180 (100), 95 (79).
Preparation of Phidolopin (36)

Deprotection of the MOM derivative was achieved by refluxing 54 (.025 g, .067 mmol) dissolved in chloroform (3 ml) in 50% acetic acid plus one drop of concentrated sulphuric acid for 1 hour. Progress of the reaction was monitored via TLC (ethyl acetate). The yellow reaction mixture was partitioned between water (10 ml) and chloroform (4 x 20 ml). The chloroform layer was dried over sodium sulphate and filtered. Evaporation of the solvent in vacuo, followed by purification by preparative TLC (ethyl acetate) gave .0215 g (97%) of Phidolopin (Rf .19), yellow needles: mp 212-213°C (CHCl3); 1H NMR (80 MHz, CDCl3-DMSO-d6) δ 3.41 (s, 3H), 3.59 (s, 3H), 5.48 (s, 2H), 7.16 (bd, H = 8.5 Hz, 1H), 7.63 (dd, J = 1.9, 8.5 Hz, 1H), 7.65 (s, 1H), 8.06 (d, J = 1.9 Hz, 1H), 10.6 (s, 1H) ppm; HRMS, m/z observed 331.0908, required for C14H13N5O5 331.0917; EI-LRMS m/z (relative intensity) 331 (M+, 91), 313 (20), 180 (100), 152 (29), 123 (21), 95 (30); UV (CHCl3) 355 (ε 1900), 275.8 (ε 9100); 13C NMR (750 MHz, CDCl3-DMSO-d6) δ 27.62, 29.46, 47.99, 105.89, 119.71, 124.94, 127.88, 135.48, 142.21, 148.58, 151.11, 152.56, 154.57 ppm.

Preparation of MOM desmethylphidolopin 56

Purified N-bromosuccinimide (1.470 g, 8.25 mmol) was added to a refluxing solution of 42 (.41 g, 2.1 mmol) dissolved in carbon tetrachloride (5 ml) in a 10 ml round bottom flask. The refluxing
mixture was irradiated for 90 minutes with a 250 W sun lamp. After cooling, the mixture was filtered and the filtrate was concentrated in vacuo. The resulting brown solid was dissolved in tetrahydrofuran (5 ml) and added dropwise to a solution of 3-methylxanthine (.3 g, 1.8 mmol) and 0.1 M NaOH (3.5 ml) which was stirred at room temperature for 16 hours. At the end of this time, the reaction mixture was partitioned between distilled water (15 ml) and chloroform (4 x 25 ml). The chloroform layer was dried over anhydrous Na$_2$SO$_4$ and filtered. Evaporation of the solvent followed by purification by radial preparative TLC (ethyl acetate) gave 0.026 g (25%) of pure 56 as a white solid: $^1$H NMR (80 MHz, CDCl$_3$) $\delta$ 3.35 (s, 3H), 3.44 (s, 3H), 5.44 (s, 2H), 7.38 (d, $J$ = 9 Hz, 1H), 7.66 (dd, $J$ = 9, 2 Hz, 1H), 7.95 (d, $J$ = 5 Hz, 1H), 8.26 (s, 1H) ppm; HRMS, m/z observed 361.1025, required for C$_{15}$H$_{15}$N$_5$O$_6$ 361.1495; EI-LRMS m/z (relative intensity) 361 (76), 343 (31), 329 (46), 299 (38), 166 (75), 152 (34), 134 (61), 121 (30), 105 (38), 94 (63), 77 (95), 51 (100).

Preparation of Desmethylphidolopin (37)

Deprotection of the MOM derivative was achieved by refluxing 55 (0.026 g, 0.071 mmol) dissolved in chloroform (3 ml) in 50% acetic acid plus one drop of concentrated sulphuric acid for 1 hr. Progress of the reaction was monitored via TLC (ethyl acetate). The yellow reaction mixture was partitioned between water (10 ml) and chloroform (4 x 20 ml). The chloroform layer was dried over anhydrous Na$_2$SO$_4$ and filtered.
Evaporation of the solvent in vacuo, followed by purification by preparative TLC (ethyl acetate) gave .024 g (94%) of desmethyldoloplin (37) (R_f .15) as a yellow solid: ^1^H NMR (80 MHz, CDCl_3) δ 3.46 (s, 3H), 5.45 (s, 2H), 7.10 (d, J = 8.5 Hz, 1H), 7.68 (dd, J = 8.5, 1.9 Hz, 1H), 7.82 (s, 1H), 8.05 (d, J = 1.9 Hz, 1H), 11.03 (s, 1H); HRMS, m/z observed 317.0760, required for C_{13}H_{11}N_{5}O_{5} 317.0761; EI-LRMS m/z (relative intensity) 317 (M^+, 2), 299 (10), 166 (100), 152 (49), 123 (45), 106 (37), 95 (55), 77 (53), 68 (80); ^1^C NMR (75 MHz, CDCl_3-DMSO-d_6) δ 28.5, 47.9, 106.3, 119.9, 125.2, 127.6, 135.2, 142.3, 150.4, 151.1, 152.8, 154.9 ppm.
Collection Data

Bryozoans were collected using SCUBA at various locations in Barkley Sound, B.C. at depths from 2 to 10 m. Immediately after collection, the whole animals were immersed in methanol and stored for one to three days at room temperature. If the animals were not immediately worked up, they were stored at lower temperature (about 2°C) until used, normally within one month.

Extraction and Chromatographic Separation

1. *Diaperoecia californica* (d'Ornigny 1892)

*Diaperoecia californica* (653 g dried weight after extraction) was ground in a Waring blender with the methanol (1 L) used for extraction of the whole animals. The suspension of ground bryozoans was filtered through celite, in vacuo, yielding a reddish brown aqueous methanolic filtrate which was concentrated to approximately 300 ml and partitioned between brine (200 ml) and ethyl acetate (3 x 400 ml). The combined dark red ethyl acetate layers were dried over anhydrous Na₂SO₄. Filtration, followed by evaporation, in vacuo, gave 5.8 g (.89%) of a dark red crude oil. Flash chromatographic fractionation (40 mm diameter
column, 15 cm silica gel, step gradient 5% ethyl acetate/hexanes to 100% ethyl acetate) yielded fractions containing fats, sterols, pigments as well as three strongly absorbing UV bands on TLC (100% ethyl acetate). Purification of the most non polar band by column chromatography, Sephadex LH-20 (7:3 methanol/chloroform) yielded a strongly retained yellow band corresponding to 4-hydroxymethyl-2-nitrophenol (38) (4.5 mg, .007%, Rf. 35). Column chromatography of the next most polar fraction using Sephadex LH-20 (7:3 methanol/chloroform) afforded a strongly retained yellow band corresponding to phidolopin (36) (1.0 mg, <.001%, Rf. 24). Purification of the most polar band in a similar fashion gave desmethylphidolopin (37) (3.7 mg, <.001%, Rf. 15).

Phidolopin (36): mp 211-212°C (CHCl₃); UV (CH₃CN) λₘₐₓ 351 nm (ε 3300), 275 (ε 16800); ¹H NMR (270 MHz, CDCl₃) δ 10.55 (s, 1H, exchanges with D₂O), 8.09 (d, J = 2.5 Hz, 1H), 7.63 (s, 1H), 7.61 (dd, J = 2.5, 8.5 Hz, 1H), 7.17 (d, J = 8.5 Hz, 1H), 5.46 (s, 2H), 3.59 (s, 3H), 3.40 (s, 3H); HRMS observed m/z 331.0914, required for C₁₄H₁₃N₅O₅ 331.0917; LRMS, m/z (relative intensity) 331 (14), 313 (25), 180 (100), 152 (38).

Desmethylphidolopin (37): ¹H NMR (80 MHz, CDCl₃) δ 10.60 (s, 1H), 8.06 (d, J = 2 Hz), 7.63 (s, 1H), 7.59 (dd, J = 2, 9 Hz), 7.13 (d, J = 9 Hz), 5.39 (s, 2H), 3.51 (s, 3H) ppm; HRMS observed m/z 317.0760, calculated for C₁₃H₁₁N₅O₅ 317.0761; LRMS, m/z (relative intensity) 317 (4), 299
4-hydroxymethyl-2-nitrophenol (38): $^1$H NMR (80 MHz, CDCl$_3$) $\delta$ 10.53 (s, 1H), 8.09 (d, $J$ = 2 Hz), 7.58 (dd, $J$ = 2, 9 Hz, 1H), 4.69 (s, 2H) ppm; HRMS m/z 169.0375, calculated for C$_7$H$_7$NO$_4$ 169.0375; LRMS, m/z (relative intensity) 169 (100), 152 (11), 123 (25), 122 (11), 106 (16), 95 (15), 77 (25), 65 (45).

4-methoxymethyl-2-nitrophenol (39): $^1$H NMR (80 MHz, CDCl$_3$) $\delta$ 10.58 (s, 1H), 8.10 (d, $J$ = 2 Hz, 1H), 7.60 (dd, $J$ = 2, 9 Hz, 1H), 4.43 (s, 2H), 3.44 (s, 3H) ppm; HRMS observed m/z 183.0535, calculated for C$_8$H$_9$NO$_4$ 183.0532; LRMS, m/z (relative intensity) 183 (59), 182 (29), 152 (100), 141 (25), 136 (21), 123 (12), 106 (39), 105 (14), 77 (24), 65 (12).

2. The remaining bryozoans, *Heteropora alaskensis*, *Hippodiplosia insculpta*, *Tricellaria ternata*, were extracted as described above for *D. californica*. The results are shown in the Table 13.
Table 13: Nitrophenols from Northeast Pacific Bryozoans

<table>
<thead>
<tr>
<th>Organism</th>
<th>Crude EtOAc Extract</th>
<th>Yield mg (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>36</td>
<td>37</td>
</tr>
<tr>
<td>1 Diaperoecia californica</td>
<td>5.8 ( .89)</td>
<td>1.0 ( .001)</td>
</tr>
<tr>
<td>2 Heteropora alaskensis</td>
<td>5.7 (1.20)</td>
<td>-</td>
</tr>
<tr>
<td>3 Hippodiplosia insculpta</td>
<td>3.7 (1.50)</td>
<td>-</td>
</tr>
<tr>
<td>4 Tricellaria ternata</td>
<td>5.2 ( .72)</td>
<td>-</td>
</tr>
</tbody>
</table>

* g (* dry weight after extraction)
Collection Data

*Anthoarcuata graceae* was collected during all seasons at various locations in Barkley Sound, B.C. at depths of 1 to 5 metres. Immediately after collection, the sponge was immersed in methanol or ethanol and stored at room temperature for up to three days. If the sponge was not worked up immediately, it was stored at low temperature (4-(-5)°C) until used (typically within 2 weeks).

Extraction and Chromatographic Separation

During the course of study on the extracts of the marine sponge *Anthoarcuata graceae*, a number of collections were made yielding little or no observed variation in metabolites. Therefore, the following represents a typical procedure.

After storage at room temperature for 2 days, the aqueous methanolic layer was decanted and stored at room temperature while the sponge, approximately 1200 g (dry weight after extraction) was further soaked in dichloromethane (6 L) for 12 hours before being transferred in dichloromethane into a Waring blender and ground down into a slurry. This crude slurry was vacuum filtered, along with the initial aqueous methanolic layer, through celite and the deep red filtrate was concen-
treated to about 250 ml before being partitioned between brine (15 ml) and dichloromethane (4 x 30 ml). The combined dichloromethane extracts were then dried over anhydrous Na$_2$SO$_4$. The sponge solids from the filtration were transferred back into the original collection jars and soaked in dichloromethane three more times at 1 day intervals. The same procedure of filtration, partitioning of filtrate and drying the organic layers over anhydrous Na$_2$SO$_4$ was repeated each day.

The combined dichloromethane extracts were filtered and concentrated in vacuo to yield a crude dark red oil, 46.9 g (3.9% of dry sponge after extraction). The oil was fractionated by flash chromatography (50 mm diameter column, 16 cm silica gel, step gradient of 100% hexanes to 30% ethyl acetate/hexanes) to yield crude fractions containing fats, pigments, carotenoids and a mixture of steroids detected by analytical TLC (50% ethyl acetate/hexanes). The combined steroid containing fractions (charring a bright pink colour with 50% H$_2$SO$_4$ spray reagent) were pooled and evaporated to yield 8.2 g (.68%) of a still crude red oil. Further purification of this complex mixture of fats, carotenoids and pigments in addition to the steroidal components was carried out using repeated column chromatography (20 mm diameter column, packed with 1 m of Sephadex LH-20, 55% methanol/chloroform as running solvent) to yield fractions containing fats, carotenoids and steroids. The still impure steroidal fraction from column chromatography was further purified by preparative reverse phase HPLC (10% water/acetonitrile) to yield two major peaks (retention times 40.6 min. and 47.4 min., flow rate of 2.8 ml/min., UV $\lambda_{max}$ 254 nm) which each were found to possess 3 novel minor steroids as detected by analytical TLC analysis.
Final separation and purification of the three components from each HPLC peak were carried out by preparative TLC (5% methanol/ dichloromethane) yielding compound A (0.0064 g, 0.0005%, R_f 0.74), compound D (0.0073 g, 0.0006%, R_f 0.64), compound F (0.0037 g, 0.0003%, R_f 0.42) from the second peak with retention time 47.4 min.

Compound 116: mp 92-93°C (CH3CN); UV 248.9 nm (ε 11600); IR (CHCl3 cast) 3031, 2956, 2871, 1686, 1643, 1606 cm⁻¹; 1H NMR (270 MHz, CDCl3) δ 0.73 (s, 3H), 0.95 (d, J = 6.0 Hz, 3H), 1.17 (s, 3H), 1.61 (s, 3H), 1.69 (s, 3H), 5.09 (t, J = 6.4 Hz, 1H), 6.19 (s, 1H) ppm; 13C NMR (75 MHz, CDCl3) δ 202.36, 199.56, 161.06, 127.09, 125.48, 124.99, 56.55, 55.91, 50.99, 46.83, 42.57, 39.14, 35.98, 35.56, 35.50, 34.22, 34.17, 33.98, 27.99, 25.74, 24.69, 23.98, 20.89, 18.55, 17.51, 11.91 ppm; EI-HRMS, m/z observed 396.3034, required for C27H40O2 396.3030; EI-LRMS m/z (relative intensity) 396 (26), 381 (19), 312 (68), 283 (46), 270 (13), 257 (19), 243 (18), 137 (52), 105 (20), 95 (55), 81 (52), 69 (92), 55 (100).

Compound 117: mp 111-112°C (CH3CN); UV (CH3CN) 248.6 nm (ε 12500); 1H NMR (270 MHz, CDCl3) δ 0.73 (s, 3H), 0.85 (d, J = 7.0 Hz, 3H), 1.02 (d, J = 8.0 Hz, 3H), 1.17 (s, 3H), 3.49 (s, 3H), 4.65 (s, 1H), 4.73 (s, 1H), 6.17 (s, 1H) ppm; 13C NMR (75 MHz, CDCl3) 202.36, 199.56, 161.08, 156.65, 125.44, 106.06, 56.61, 55.77, 50.93, 46.79, 42.55, 39.10, 35.62, 35.51, 34.50, 34.18, 33.96, 30.89, 29.70, 27.98, 23.95, 22.70, 21.99, 21.84, 20.86, 18.62, 17.51, 11.88 ppm; EI-HRMS, m/z observed 410.3182, required for C28H42O2 410.3187; EI-LRMS, m/e
Compound 118: mp 142-143°C (CH₃CN); UV (CH₃CN) \( \lambda_{\text{max}} \) 250.2 nm (\( \epsilon \) 12500); IR (CHCl₃ cast) 3552, 3021, 2951, 2869, 1744, 1717, \( 1648 \) cm\(^{-1} \); ¹H NMR (400 MHz, CDCl₃) \( \delta \) 0.73 (s, 3H), 0.96 (d, J = 6.7 Hz, 3H), 1.21 (s, 3H), 1.62 (s, 3H), 1.62 (s, 3H), 1.69 (s, 3H), 1.88 (m, 1H), 2.08 (d, J = 13.3 Hz, 1H), 2.19 (d, J = 13.3 Hz, 1H), 2.41 (m, 1H), 3.79 (s, 3H), 5.10 (t, J = 8.0 Hz, 1H), 6.73 (t, J = 3.3 Hz, 1H) ppm; ¹³C NMR (75 MHz, CDCl₃) \( \delta \) 200.91, 173.37, 145.10, 136.28, 131.05, 125.09, 79.90, 56.26, 56.02, 55.91, 53.39, 49.66, 42.94, 39.92, 39.35, 36.06, 35.55, 32.32, 32.21, 28.11, 25.75, 24.74, 24.35, 22.11, 21.79, 18.66, 17.66, 12.03 ppm; EI-HRMS, m/z observed 442.3083, required for C$_{28}$H$_{42}$O$_{4}$ 442.3079; EI-LRMS m/z (relative intensity) 442 (79), 427 (24), 383 (10), 358 (23), 340 (18), 329 (41), 311 (19), 298 (30), 283 (7), 269 (11), 135 (32), 147 (22), 107 (41), 95 (58), 81 (49), 69 (100), 55 (68).

Compound 119: Needles; mp (CH₃CN) 155-157°C; UV (CH₃CN) \( \lambda_{\text{max}} \) 249.3 (\( \epsilon \) 12034); IR (CHCl₃, cast), 3022, 2929, 2856, 1744, 1717, 1643 cm\(^{-1} \); ¹H NMR (400 MHz, CDCl₃) \( \delta \) 0.73 (s, 3H), 0.97 (d, J = 6.5 Hz, 3H), 1.04 (d, J = 6.5 Hz, 3H), 1.20 (s, 3H), 1.89 (m, 1H), 2.10 (d, J = 13.5 Hz, 1H), 2.18 (d, J = 13.5 Hz, 1H), 2.39 (m, 1H), 3.77 (s, 3H), 4.65 (s, 1H), 4.71 (s, 1H), 6.73 (t, J = 2.5 Hz, 1H) ppm; ¹³C NMR (75 MHz, CDCl₃) \( \delta \) 200.91, 173.36, 156.79, 145.08, 136.29, 106.01, 79.89, 56.25, 55.93, 53.40, 49.64, 46.68, 42.95, 39.93, 39.34, 35.72, 34.64, 33.81, 32.32, 32.21, 30.99, 28.13, 24.34, 22.11, 22.01, 21.87, 21.79, 18.74, 12.03.
ppm; EI-HRMS, m/z observed 456.3236, required for C_{29}H_{44}O_{4} 456.3241; EI-LRMS m/z (relative intensity) 456 (2), 396 (2), 367 (23), 353 (5), 298 (48), 284 (10), 269 (65), 147 (35), 124 (56), 109 (49), 95 (73), 81 (68), 69 (89), 55 (100).

Compound 120: _1^H NMR (400 MHz, CDCl\textsubscript{3}) \delta 0.73 (s, 3H), 0.93 (d, J = 6 Hz, 3H), 1.28 (d, J = 8 Hz, 3H), 1.52 (s, 3H), 1.69 (s, 3H), 2.18 (d, J = 16 Hz, 1H), 2.25 (d, J = 16 Hz, 1H), 3.29 (s, 3H), 4.52 (t, J = 3 Hz, 1H), 5.09 (t, J = 7 Hz, 1H), 5.28 (s, 1H) ppm; EI-HRMS, m/z observed 414.3149, required for C_{27}H_{42}O_{3} 414.3136; EI-LRMS m/z (relative intensity) 414 (19), 382 (28), 268 (10), 330 (16), 298 (15), 269 (29), 245 (30), 161 (26), 138 (34), 109 (34), 97 (52), 81 (49); 69 (100), 55 (81); UV 264.7 nm (\epsilon 10971, c = .04) + NaOH (304.7 nm, \epsilon 9731).

Compound 121: _1^H NMR (400 MHz, CDCl\textsubscript{3}) \delta 0.73 (s, 3H), 0.94 (d, J = 8 Hz, 3H), 1.03 (d, J = 7 Hz, 6H), 1.31 (s, 3H), 2.17 (d, J = 16 Hz, 1H), 2.29 (d, J = 16 Hz, 1H), 3.33 (s, 3H), 4.57 (t, J = 2 Hz, 1H), 4.69 (s, 1H), 4.76 (s, 1H), 5.28 (s, 1H) ppm; EI-HRMS, m/z observed 428.3284 required for C_{28}H_{40}O_{3} 428.3291; EI-LRMS m/z (relative intensity) 428 (5), 396 (13), 344 (12), 312 (14), 300 (12), 271 (24), 161 (30), 138 (37), 107 (38), 95 (52), 81 (61), 69 (95), 55 (100).
Preparation of 4-ene-3,6-dione 122 by Jones oxidation

The Jones reagent was prepared by dissolving chromium trioxide (26.72 g, 270 mmol) in 50 ml distilled water in a 100 ml beaker. The beaker was immersed in an ice water bath and concentrated sulfuric acid (911.5 ml, 200 mmol) was added followed by enough distilled water to bring the total volume to 100 ml. A solution of cholesterol (1.00 g, 2.6 mmol dissolved in 30 ml acetone was stirred in a 50 ml round bottom flask at 0°C in an ice water bath before 2 ml of the previously prepared cold oxidation reagent was added at a rate to maintain a reaction mixture temperature of around 20°C. The stirring was maintained for 3 hours, with the reaction mixture turning yellowish from the original deep red colour. After 3 hours, 10 ml of methanol was added to destroy excess reagent, and the reaction mixture was filtered through celite. The resulting light brown solution was placed into a 150 ml separatory funnel and extracted with chloroform (4 x 50 ml). The organic layer was further washed with 50 ml of brine and dried over anhydrous magnesium sulfate. Filtration, followed by evaporation in vacuo yields a light brown gum (462 mg) which on preparative TLC yielded one highly absorbing UV band at R_f 0.50 (30% ethyl acetate/hexanes) which based on spectral data corresponded to the ene-dione 122, 337.9 mg (32.7%).

Compound 122: mp 132-134°C (CH_3CN); IR (CHCl_3 cast) 2953, 2871, 1685, 1606 cm⁻¹; H NMR (270 MHz, CDCl_3) δ 0.74 (s, 3H), 0.87 (d, J = 6 Hz, 6H), 0.94 (d, J = 6 Hz, 3H), 1.19 (s, 3H), 6.19 (s, 1H) ppm; C NMR (75 MHz, CDCl_3) δ 202.25, 199.40, 160.98, 125.33, 56.42, 55.82,
50.83, 46.71, 39.70, 39.36, 39.01, 35.57, 35.40, 34.09, 33.87, 27.91, 23.86, 23.69, 22.75, 20.77, 18.55, 17.40, 11.79 ppm; EI-HRMS, m/z observed 398.3186 required for C_{27}H_{42}O_{2} 398.3187; EI-LRMS m/z (relative intensity) 392 (2), 383 (14), 370 (6), 343 (2), 329 (6), 243 (40), 147 (24), 135 (33), 124 (35), 105 (37), 91 (65), 79 (64), 67 (55), 55 (100).

Anthosterone B-acetate (123)

2.8 mg (0.006 mmol) of Compound D 119 was stirred in pyridine and acetic anhydride (2:1) in a 10 ml round bottom flask for 12 hours at room temperature. Evaporation of the solvent and excess reagent gave acetylated product 123, as a white solid, in quantitative yield.

Compound 123: \(^1\)H NMR (270 MHz, CDCl\(_3\)) \(\delta\) 0.72 (s, 3H), 0.97 (d, J = 8 Hz, 3H), 1.03 (d, J = 8 Hz, 6H), 1.10 (s, 3H), 2.16 (s, 3H), 2.47 (d, J = 14 Hz, 1H), 2.65 (d, J = 15 Hz, 1H), 3.76 (s, 3H), 4.67 (s, 1H), 4.73 (s, 1H), 6.81 (t, J = 4 Hz, 1H) ppm; EI-HRMS, m/z observed 498.3347 required for C_{31}H_{46}O_{5} 498.3347; EI-LRMS m/z (relative intensity) 498 (1), 483 (2), 456 (37), 382 (4), 312 (28), 287 (27), 135 (28), 123 (27), 109 (36), 95 (57), 81 (57), 69 (93), 55 (100).
Bioassay results for phidolopin (36) and desmethylphidolopin (37)

Phidolopin (36) and desmethylphidolopin (37) demonstrated significant levels of activity in the standard disk minimum inhibitory concentration bioassays performed in our laboratory. Phidolopin showed in vitro antifungal activity with a minimum inhibitory concentration against *Pythium ultimum* (75 ug/.25 in disk), *Helminthosporium satium* and *Rhizoctonia solani* (70 ug/.25 in disk) and antibacterial activity against *Bacillus subtilis* 19 ug/.25 in disk) and *Staphlococcus aureus* (75 ug/.25 in disk).

Desmethylphidolopin (37) showed in vitro antifungal activity against *Pythium ultimum* (22.5 ug/.25 in disk) as well as antimicrobial activity against *Bacillus subtilis* (22.5 ug/.25 in disk).
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