Characterization of Secondary Metabolites From Caribbean Gorgonians and Sponges

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ABSTRACT

Characterisation of Secondary Metabolites from Caribbean Gorgonians and Sponges

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Part I of this thesis deals with the chemistry of Caribbean gorgonians. Chapter I reviews the chemistry of the genus *Pseudo terogorgia*, while chapters 2 - 5 describes the investigation of selected *Pseudopterogorgia* species. In chapter 2, the investigation of *P. rigida* and *P. blacquillensis* resulted in the isolation of the known bisabolane derivatives curcuphenol and curcuquinone, and 12-hydroxy-*E*-γ-bisabolene, respectively. Chapter 3 describes the isolation of the germacrane derivatives furanodiene and neosericenine, and the elemane derivatives isosericenine and anhydrosericealactone from a *Pseudopterogorgia* species. This appears to be the first reported occurrence of anhydrosericealactone in nature. Next, a reinvestigation of *P. americana* is described (chapter 4). This resulted in the isolation of furanodiene and a number of related germacrane derivatives. These included (+)-germacrene-D and isofuranotriene. The structure of furanotriene, previously isolated from *P. americana* was revised. Chapter 5 comprises the isolation of two new diterpenoids, designated compounds F and G, one a pseudopterane, and the other a cembrane derivative, respectively.

Four further cembrane derivatives were isolated this time from a *Plexaura* species (chapter 6). Chapter 7 reviews the cembrane derivatives isolated from marine invertebrates.

Part II describes the isolation of secondary metabolites from Caribbean sponges. An unknown sponge yielded the newly discovered 24-methylene-25-methyl-cholesterol, in addition to fucosterol. Next, the isolation of dimethyloxyaaptamine from *Aaptos aaptos* is dealt with (chapter 2). This is followed by the final chapter which consists of the isolation and characterisation of the cyclodepsipeptides, geodialactone-A and -B from a *Geodia* species. This represents the first reported occurrence of cyclodepsipeptides from sponges.

ACKNOWLEDGEMENTS

The author wishes to express his sincere gratitude to Professor W.R. Chan, under whose guidance and supervision this work was carried out. Also, to Dr. B.S. Mootoo and Dr. C.E. Seaforth, for the interest they showed in this work, and for many pleasant discussions.

My sincere appreciation and thanks to all the members of the Department of Chemistry.

I wish to express my indebtedness to Dr. P. Manchand of Hoffmann-La Roche Inc., New Jersey, for his keen interest in this work, and for providing the high resolution ^{1}H and ^{13}C NMR, mass spectra, and X-ray crystallographic data included in this thesis, unless otherwise stated. Thanks are also due to Dr. B.A. Burke of the ARCO Plant Cell Research Institute, California, who kindly provided the high resolution ^{1}H and ^{13}C NMR, and mass spectra included in Part II, chapters 1 and 2 of this thesis. Prof. J.C. Vederas of the University of Alberta, Edmonton, kindly provided the high resolution spectral (^{1}H, ^{13}C NMR and MS) data on compound F (11,12-deoxypseudopterolide) in chapter 5 (Part I). The low resolution mass spectral data included in Part I, chapters 3 and 4 was kindly provided by Dr. Ruth Moore of the Caribbean Industrial Research Institute (CARIRI), St. Augustine.

Special thanks are also due to Mr. Richard Hubbard and Mr. Richard Laydoo of the Institute of Marine Affairs (IMA), for collecting the sponges, and gorgonians, respectively, investigated in this work.

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CHAPTER SIX

An Investigation into the Constituents of *Plexaura* sp.

DEDICATED

to

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PART ONE

STUDIES OF CARIBBEAN GORGONIANS

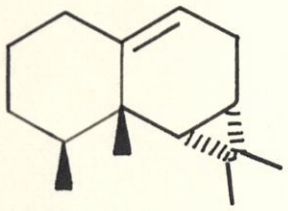
CHAPTER ONE

A REVIEW OF THE CHEMISTRY OF THE GENUS *PSEUDOPTEROGORGIA*

1.1 INTRODUCTION

Octocorals (Phylum Cnidaria) are sedentary, colonial invertebrates, that account for about 38% of the living organisms found on West Indian shallow-water reefs. Almost all these corals belong to the two families Gorgoniidae and Plexauridae. There are about 37 species belonging to the family Gorgoniidae, of which 12 species are found in the genus *Pseudopterogorgia*.[1]

Since most *Pseudopterogorgia* spp. closely resemble each other,[2] their identification can be challenging. Within recent times, there has been considerable interest in the use of chemical methods[3,4] in octocoral classification. The present review brings together, the existing literature on the secondary metabolites from the genus *Pseudopterogorgia*.



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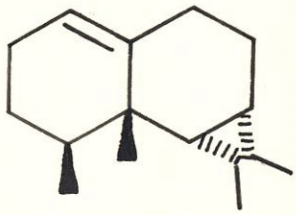

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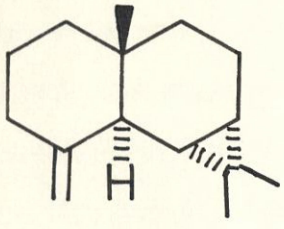

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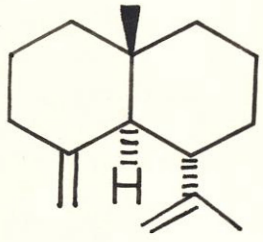

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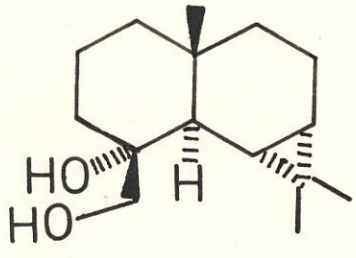


(V)

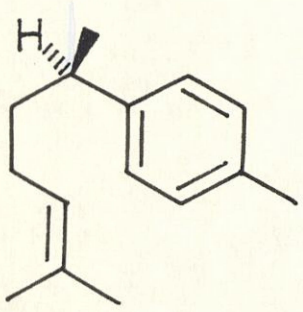
1.2 SESQUITERPENE HYDROCARBONS

Pseudopterogorgia americana (Gmelin) was one of the first gorgonian corals to be studied chemically. From the non-saponifiable fraction of a hexane extract of *P. americana*, Weinheimer and his co-workers[5] isolated the known double-bond isomers 9-aristolene (I) and 1(10)-aristolene (II). These compounds were accompanied by (+)-γ-maaliene (III), and the nonisoprenoid (+)-β-gorgonene (IV). All four compounds were isolated after chromatography on silver nitrate impregnated silica.

The structures of the aristolenes were determined by comparison with literature data, particularly their ^{1}H NMR spectra.[6] (+)-γ-Maaliene (III) was identified after conversion (osmium tetroxide) to the diol (V). (+)-β-Gorgonene (IV) was isolated both in the free state and as the crystalline silver nitrate complex. The structure was determined using X-ray diffraction.

1.3 BISABOLANE DERIVATIVES

McEnroe and Fenical[2] isolated four bisabolane derivatives from *P. rigida*. Column chromatography of a crude extract of *P. rigida* gave an initial fraction which was shown to be a mixture of the α-, β- and γ-bisabolanes, and α-curcumene (VI), based on GC-MS characteristics. From



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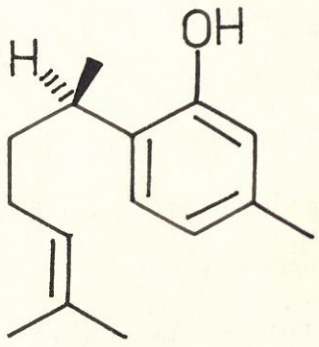

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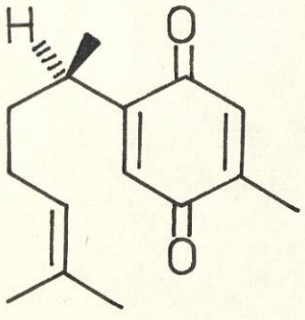

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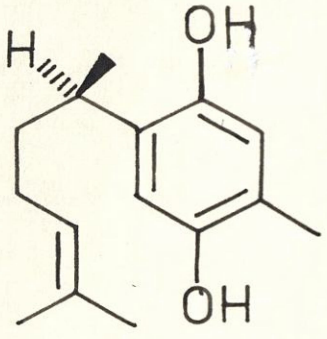

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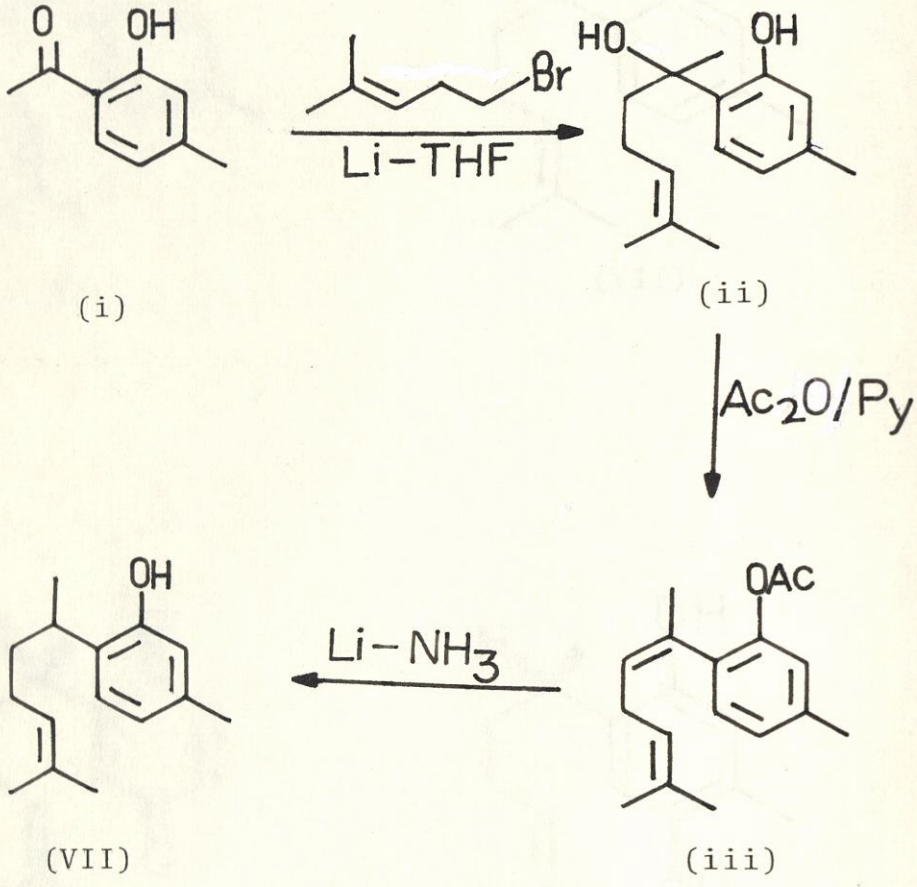
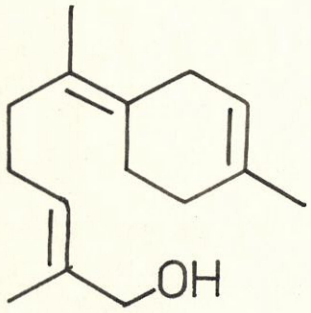


SCHEME I

more polar fractions (-)-curcuphenol (VII), (-)-curcuquinone (VIII), and (-)-curcuhydroquinone (IX) were isolated, all were shown to possess modest antibacterial activity.

Curcuphenol (VII) was identified on the basis of spectroscopic data and chemical synthesis (Scheme I). Condensation of 2-hydroxy-4-methylacetophenone (i) with 5-bromo-2-methyl-2-pentene gave the benzyl alcohol (ii) in 90% yield. The benzylic hydroxy group in (ii) was found to be resistant to dehydration with strong acids. Base-catalyzed acetylation resulted in facile dehydration of the tertiary alcohol to give (iii) in quantitative yield. Reduction of (iii) with lithium in liquid ammonia gave a 97% yield of racemic curcuphenol (VII). The absolute stereochemistry at the lone asymmetric carbon of natural (VII) was established as *R* by reduction of the mesylate with lithium in ammonia to obtain the optically active parent hydrocarbon (VI), which showed identical optical rotation to previously isolated material.

Curcuquinone (VIII) was isolated as a viscous yellow oil which showed an infrared (IR) absorption at 1650 cm^{-1} ($\nu_{C=O}$), due to a 1,4-quinone, and an ultra violet (UV) absorption maximum at 253 nm (ε 10,200). The molecular formula $C_{15}H_{20}O_2$ was established by high resolution mass spectrometry. The ^1H NMR spectrum of (VIII) indicated close similarities to (VII) except for one less aromatic proton in (VIII).



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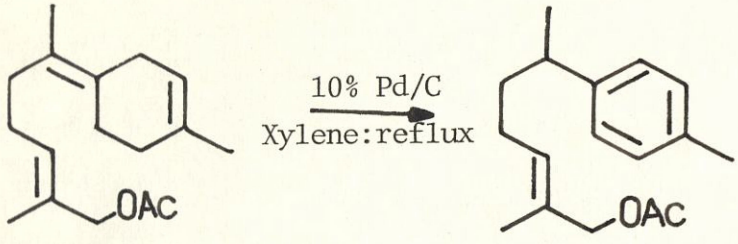
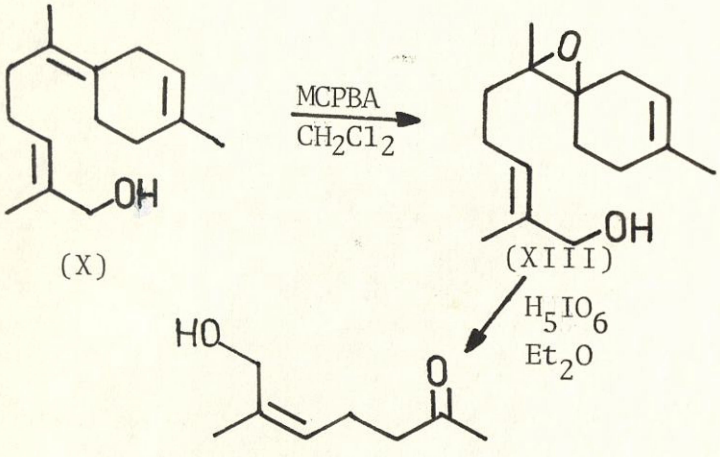


(XI) (XII)



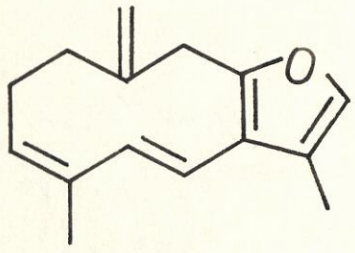
(X) (XIII)

(XIV)

SCHEME II

Curcuhydroquinone (IX), a colourless viscous oil, showed a strong hydroxyl band in its IR spectrum at 3250 cm^{-1}. The molecular formula $C_{15}H_{22}O_2$ for (IX), was established by high resolution mass spectrometry. The ^1H NMR spectrum of (IX) was interpreted in terms of the hydroquinone structure. Oxidation of (IX) with Jones' reagent led to (-)-curcuquinone (VII). The absolute configuration of (VIII) and (IX) was established by chemical correlation with (-)-α-curcumene (VI).

From a gorgonian belonging to the genus *Pseudopterogorgia*, Look, Buchholz, and Fenical[7] recently reported the structure of a new sesquiterpene alcohol (X). Alcohol (X) was determined to be 12-hydroxy-*E*-γ-bisabolene on the basis of spectroscopic and chemical evidence. Treatment of the acetate (XI) with 10% palladium on carbon in refluxing xylene for 50 hours, yielded the aromatized curcumene derivative (XII) as the major reaction product. Further information on the structure (X) was obtained by selective cleavage of the compound at the tetrasubstituted double bond. Thus, treatment of (X) with stoichiometric amounts of m-chloroperbenzoic acid in disodium hydrogenphosphate-buffered methylene chloride yielded the epoxide (XIII) in 93% yield. Cleavage of epoxide (XIII) with periodic acid in diethyl ether gave a complex mixture from which keto-alcohol (XIV) was isolated by silica HPLC (Scheme II).



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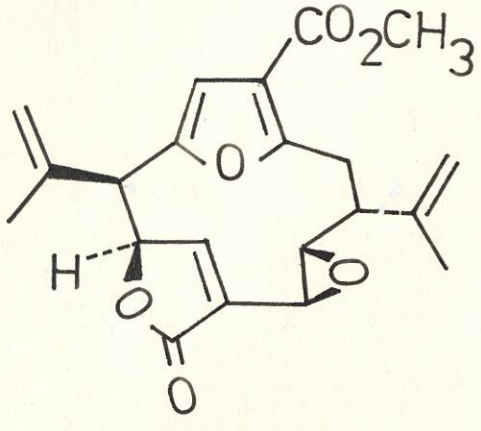


(XVI)

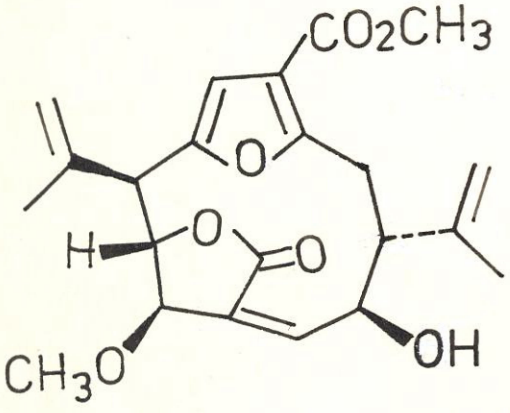
The stereochemistry at the tetrasubstituted double bond was determined to be *E*, based on comparison of the ^{13}C NMR bands of (X) with those from both *E*- and *Z*-γ-bisabolenes. The hydroxyl group at C-12 was shown to be in a *cis* relationship with the olefinic proton at C-2 on the basis of nuclear Overhauser enhancement difference spectroscopy (NOEDS).[8]

1.4 GERMACRANE DERIVATIVE

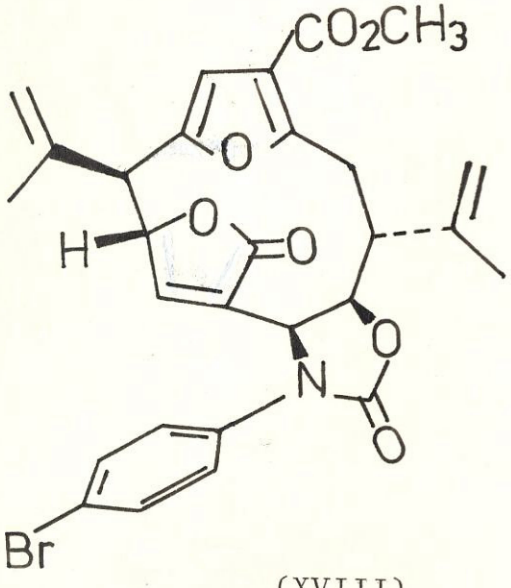
A recent report by Fenical and his co-workers[9] indicated that germcrane derivatives are widely distributed in octocorals. From *P. americana* a new, moderately stable furanogermacrene derivative (XV) was isolated, and given the trivial name furanotriene. These workers arrived at the structure indicated, on the basis of extensive decoupling experiments and the study of molecular models.

1.5 DITERPENOIDS

Fenical's group[10] recently isolated an irregular diterpenoid with cytotoxic properties from *P. acerosa*, for which they proposed the name pseudopterolide (XVI). Pseudopterolide (XVI) had the molecular formula $C_{21}H_{22}O_6$ as determined by high resolution mass spectrometry. Signals in the ^{13}C NMR spectrum at δ 163.8(s), 160.3(s), 150.4(s),



(XVII)

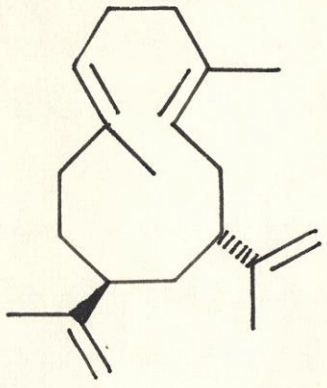


(XVIII)

111.5(d), and 51.4(q), coupled with an infrared absorption at 1712 cm^{-1} and two singlets in the ^1H NMR spectrum at δ 6.44(1H) and 3.82(3H), indicated that (XVI) possessed an α,α´,disubstituted β-carbomethoxy furan moiety. A second carbonyl absorption at 1767 cm^{-1}, along with a one-proton signal at δ 7.12 in the ^1H NMR spectrum and signals at δ 171.9(s), 151.3(d), 129.0(s), and 79.7(d) in the ^{13}C NMR spectrum were assigned to an α-substituted α,β-unsaturated γ-lactone functionality. The remaining oxygen atom in (XVI) was shown to be a *cis*-disubstituted epoxide on the basis of ^{13}C NMR signals at δ 59.9(d) and 52.5(d), and ^1H NMR signals at δ 3.65 (1H,d,J=4Hz) and 2.99 (1H,dd,J=10,4Hz).

Consideration of the olefinic and functional group unsaturation in (XVI) led to the conclusion that (XVI) was monocarbocyclic. The gross structure (XVI) was arrived at on the basis of ^1H NMR decoupling experiments and computer simulation. The complete structure and absolute configuration was determined from X-ray diffraction analysis of the crystalline urethane derivative (XVIII), derived from (XVI) by treatment with sulfuric or *P*-toluenesulfonic acid in methanol, followed by treatment of the derivative (XVII) with *P*-bromophenylisocyanate in benzene/pyridine.

The structure of pseudopterolide represents a new monocyclic skeleton related in part to cubitene (XIX),[11] a 12-membered ring diterpenoid with two isopropyl groups



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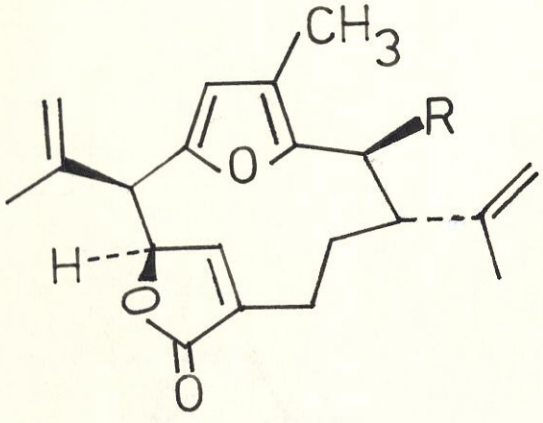
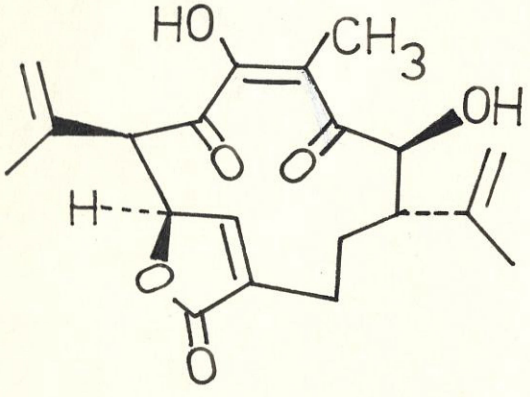


(XX) R = OH

(XXI) R = H

(XXIII) R = OAc

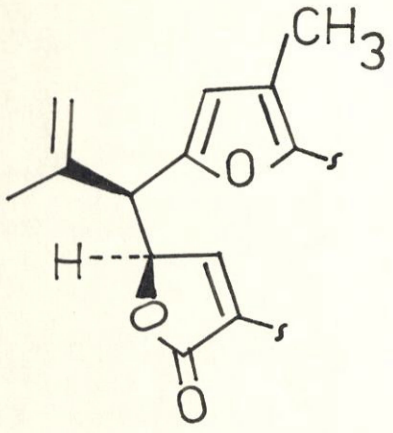


(XXII)

oriented 1,3 instead of 1,7. While (XVI) can be dissected symmetrically into two geranyl units in two possible ways, suggesting a biogenesis involving dimerisation, the authors proposed a mechanism involving ring contraction of the more prevalent 14-membered ring cembranoids.

Kallolide A - C (XX - XXII), and kallolide A acetate (XXIII) were isolated from *P. kallos* by Fenical's group.[12] They represents further members of the pseudopterane group of diterpenoids. Kallolide A, the major metabolite of *P. kallos*, possesses antiinflammatory activity against phorbol ester-induced inflammation.

The partial structure (XXIV) in (XX) was determined by spin decoupling experiments and NOEDS. The complete structure of the sole crystalline metabolite (XXIII) was determined by single crystal X-ray diffraction analysis. Structures for (XX) and (XXI) were subsequently established on the basis of spectral analysis and chemical interconversion. Thus, acetylation of (XX) gave (XXIII). On the other hand hydrogenation of (XXI) yielded the hexahydro derivative (XXV). Similarly, hydrogenation of (XX) and (XXIII) resulted in hydrogenolysis at C-2 and yielded a product in each case that was identical in all respects with (XXV) as illustrated in Scheme III.



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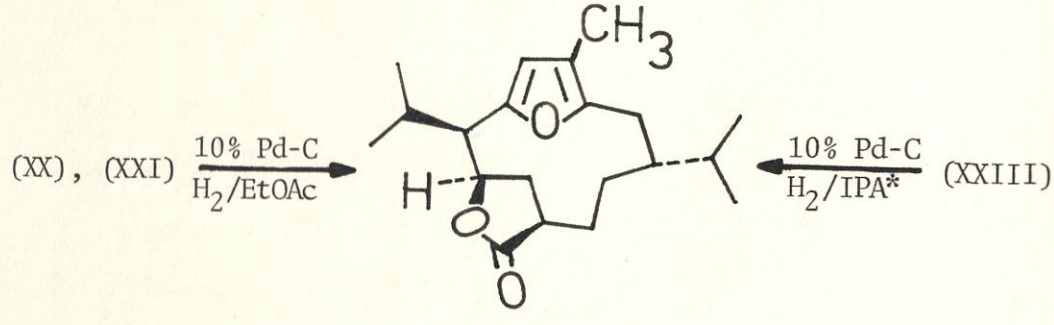
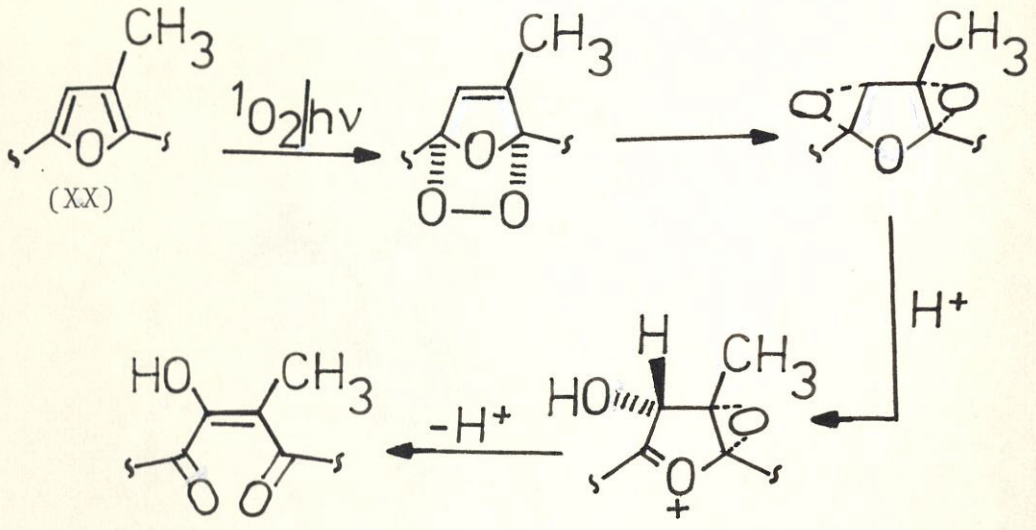


(XXV)

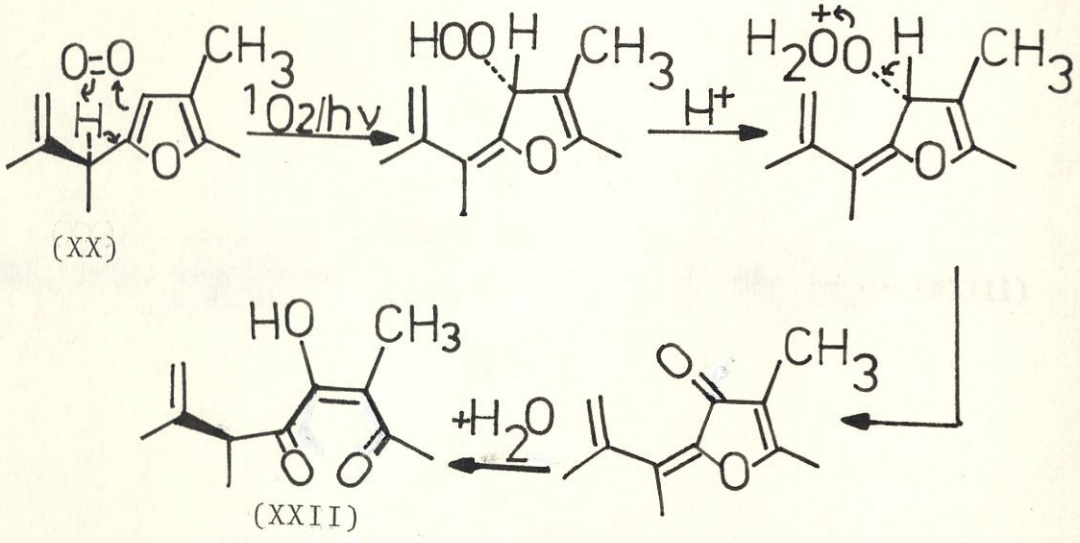
SCHEME III

*Isopropyl alcohol



(XX) (XXII)

(a) CYCLOADDITION MECHANISM



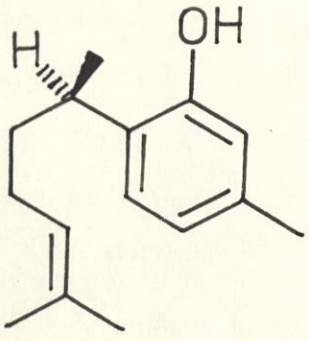
(XX) (XXII)

(b) ENE MECHANISM

SCHEME IV

Kallolide C (XXII) was isolated as a relatively unstable compound. Kallolide C (XXII) was obtained by singlet oxygen photooxidation of (XX) using polymer-bound Rose Bengal/methylene chloride/incandescent irradiation for twelve hours. While it is known that singlet oxygen reacts rapidly with furans in a $\pi^4s + \pi^2s$ cycloaddition reaction, the products are usually peroxides. Fenical's group proposed an ene mechanism (Scheme IV) to account for the formation of (XXII) from (XX). The possibility that (XXII) might be an artifact derived from (XX) was not excluded.

Although cembrane derivatives are widely distributed in octocorals (see Chapter 7), to date none has been reported from the genus *Pseudopterogorgia*. This is surprising since at least six species have so far been investigated.



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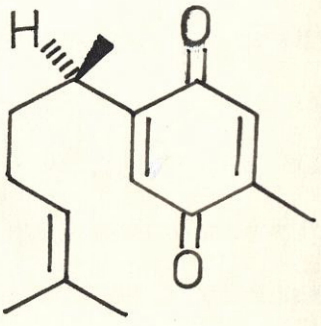

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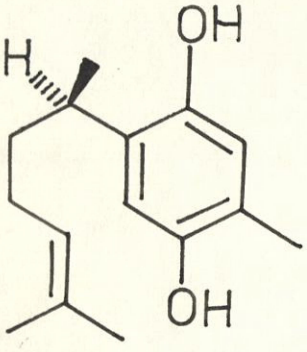

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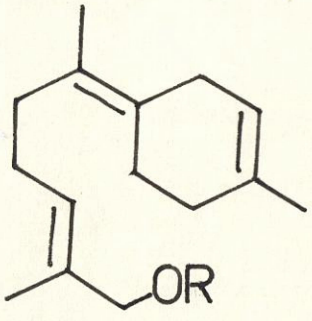


(X) R = H

(XI) R = Ac

CHAPTER TWO

CHEMOTAXONOMICAL INVESTIGATION OF THE SECONDARY METABOLITES FROM *PSEUDOPTEROGORGIA RIGIDA* AND *P. BLACQUILLENSIS* (PHYLUM CNIDARIA)

2.1 INTRODUCTION

Pseudopterogorgia rigida can be distinguished from other *Pseudopterogorgia* species because of its distinct lemon-like odor.[2] McEnroe and Fenical[2] reported the presence of the bisabolane derivatives (VII - IX) in *P. rigida*. As part of a programme to isolate and characterize secondary metabolites from Caribbean gorgonians, it was observed that *P. blacquillensis* had an odor similar to *P. rigida*, though of a lower intensity. Taxonomically *P. rigida* and *P. blacquillensis* are closer to each other than to other *Pseudopterogorgia* species.[1] To shed some light on the taxonomical relationship between *P. rigida* and *P. blacquillensis*, it was decided to make a comparative analysis of the secondary metabolites of both species.

2.2 EXTRACTION

Pseudopterogorgia rigida

Air dried specimens of *P. rigida* were extracted with acetone and the solvent evaporated to give a dark brown oil in 10% yield.

Short column chromatography of the crude extract using light petroleum (see general experimental) as mobile phase gave a fraction that consisted mainly of two components. Preparative layer chromatography (PLC hereafter) of the two component mixture using light petroleum-acetone (3:1) as eluting solvents gave, in order of increasing R_f values, curcuphenol (VII) and curcuquinone (VIII) in yields of 50% and 25% respectively (based on the crude extract).

Pseudopterogorgia blacquillensis

Air dried *P. blacquillensis* was extracted with acetone and the solvent evaporated. The crude extract was partitioned between 10% aqueous methanol and light petroleum. The aqueous methanol layer was adjusted to 30% water and extracted with dichloromethane, to give a dark brown oil on evaporation of the solvent. The dichloromethane soluble material was chromatographed on a short column using light petroleum with increasing concentrations

of ethyl acetate as mobile phase. Elution with 5% ethyl acetate in light petroleum gave a fraction which was further purified by PLC to give a colourless oil (0.4% of dried gorgonian).

2.3 ISOLATION OF KNOWN BISABOLANE DERIVATIVES

Curcuphenol (VII) was isolated as a viscous oil from *P. rigida*, $[\alpha]_D$ - 7° (c 0.9, $CHCl_3$). The UV spectrum had absorption maxima at 217 and 276 nm (ε 4200, 2700). The IR spectrum had bands at 3420 (hydroxyl), 1620, 1585 and 1510 cm^{-1} (aromatic).

The presence of a terminal isopropylidene group in (VII) was evident from ^1H NMR resonances at δ 5.10 (1H, m), 1.54 (3H,s), and 1.67 (3H,d,J=1Hz). A signal at δ 2.20 (3H,s) was assigned to an aromatic methyl, while a doublet at δ 1.22 (3H,J=7Hz) was attributed to a benzylic methyl group. An aromatic 1,2,4-trisubstituted pattern followed from resonances at δ 6.56 (1H,s), 6.70 (1H,d, J=7.5Hz), and 6.98 (1H,d,J=7.5Hz).

The above data are in agreement with the reported data[2] for curcuphenol (VII). This was supported by the ^{13}C NMR spectrum which had resonances identical with literature data for (VII).[2]

Curcuquinone (VIII), a yellow viscous oil, $[\alpha]_D$ -1.0^o (c 0.8, $CHCl_3$), had a UV maxima at 253 nm (ε 11,400). The IR spectrum of (VIII) had an absorption band at 1650 cm^{-1} (1,4-quinone).

The ^1H NMR spectrum of (VIII) was similar to that of (VII). An isopropylidene group showed resonances at δ 5.02 (1H,m), 1.54 (3H,s), and 1.64 (3H,d,J=1Hz). A benzylic methyl resonated at δ 1.11 (3H,d,J=7Hz). An aromatic methyl with a signal at δ 2.02 (3H,d,J=1.5Hz) was allylically coupled to an aromatic proton at δ 6.58 (1H,q, J=1.5Hz). Irradiation of the resonance at δ 2.02 caused the quartet at δ 6.58 to collapse into a singlet. A signal for only one more aromatic proton which resonated at δ 6.47 (1H,d,J=1Hz), was allylically coupled to a benzylic proton at δ 2.91 (1H,m).

The ^{13}C NMR spectrum of (VIII) showed signals at δ 187.4(s) and 188.5(s) attributed to the carbonyls of a 1,4-quinone. The above spectral data are in full accord with the literature data[2] for (VIII).

Curcuhydroquinone (IX), which was isolated as a major component from *P. rigida* by McEnroe and Fenical[2] was not detected in our samples; however, it might have been present as a minor component.

Chromatography of an extract of *P. blacquillensis* gave a colourless oil with IR bands at 3350 - 3450 (hydroxyl) and 1670 cm^{-1} (olefinic).

A broad signal in the ^{1}H NMR spectrum of the oil at δ 1.67 (9H,bs) was attributed to three overlapping olefinic methyls. Resonances at δ 5.30 (1H,m), and 5.37 (1H,m), were assigned to olefinic protons, while a broad singlet at δ 3.96 (2H,bs) was attributed to the carbinyl protons of a primary alcohol. The presence of the hydroxyl group was confirmed by a ^{13}C NMR resonance at δ 68.3(t), and the ready conversion to acetate (XI). The spectroscopic data suggested that the colourless oil was a γ-bisabolane derivative with one of the methyls of the isopropylidene group oxidized to a primary alcohol.

While this work was in progress, Fenical and his co-workers reported the isolation of a new γ-bisabolane derivative from a *Pseudopterogorgia* sp. The spectral data (IR, ^{1}H NMR, ^{13}C NMR) for the new compound, 12-hydroxy-*E*-γ-bisabolene, was identical with the data for the alcohol (X) isolated from *P. blacquillensis*.

The isolation of a bisabolane derivative from *P. blacquillensis* supports its taxonomical assignment by Bayer.[1]

2.4 GENERAL EXPERIMENTAL

Optical rotations were taken on a Schmidt-Haensch Polartronic-D Polarimeter and are for chloroform solutions. Ultraviolet spectra were recorded on a Perkin-Elmer 552A UV/VIS spectrophotometer and are for methanol solutions unless otherwise stated. Infrared spectra were obtained on a Pye-Unicam SP3-200 spectrophotometer. Nuclear magnetic resonance spectra were recorded on a Bruker WP80SY spectrometer and are for deuterochloroform solutions unless otherwise stated. The chemical shifts are relative to internal tetramethylsilane. Proton NMR spectra were recorded at 80.13MHz, and ^{13}C NMR spectra at 20.15MHz. Thin layer chromatography (TLC) was done on 0.25 mm thick layers of silica gel $60_{PF254+366}$ (Merck). The chromatoplates were developed over iodine vapour. Preparative layer chromatography was done on 1.0 mm thick layers of silica gel (same as for TLC). The chromatoplates were observed under a UV lamp. Short column chromatography was done using a sintered glass Buchner funnel (7.5 x 5 cm), and flask (500 mL). TLC grade silica gel was used as the stationary phase. The light petroleum used for chromatographic developments had a boiling range of 60 - 80°. High performance liquid chromatography was done on an LDC/Milton Roy HPLC system with a refractive index detector, and a Spherisorb silica column (ID 4.6 mm, length 250 mm). Melting points (Kofler apparatus) are uncorrected.

2.5 EXPERIMENTAL

Pseudopterogorgia rigida

The air dried gorgonian (225g) was extracted with acetone (2.5L) and the solvent evaporated. The extract was dissolved in 10% aqueous methanol (200 mL) and extracted with light petroleum (3 x 200 mL). The aqueous methanol layer was diluted with water (100 mL) and extracted with dichloromethane (3 x 200 mL). The solvent from the dichloromethane layer was evaporated to give an oily residue (15g, 6.7%).

Short column chromatography of the dichloromethane extract (5.7g) using light petroleum as mobile phase gave a fraction (5.0g) that consisted of mainly two components. PLC of the two component mixture (500 mg) with light petroleum-acetone (7:1) as eluting solvents, afforded, in order of increasing R_f values, curcuphenol (264 mg), and curcuquinone (111 mg).

Curcuphenol (VII) was isolated as an oil,

$[\alpha]_D$ - 7.0° : (c 0.9, $CHCl_3$)

λ_{max} : 217, 276 nm (ε 4200, 2700)

ν_{max} (neat) : 3440 (hydroxyl), 1620, 1585, 1510 (aromatic) cm^{-1}

^{1}H NMR (80MHz) : δ 1.22 (3H,d,J=7Hz), 1.54 (3H,s), 1.67 (3H,d,J=1Hz), 1.40 - 1.75 (2H,m), 1.87 (2H, bt,J=6Hz), 2.26 (3H,s), 2.95 (1H,m), 6.56 (1H,s), 6.70 (1H,d,J=7.5Hz), 6.98 (1H,d,J=7.5Hz) ppm.

^{13}C NMR (20MHz) : δ 17.7(q), 20.9(q), 21.1(q), 25.7(q), 26.1(t), 31.5(d), 37.3(t), 116.2(d), 121.7(d), 124.6(d), 126.9(d), 130.0(s), 131.9(s), 136.6(s), 152.9(s) ppm.

Curcuquinone (VIII), oil

$[\alpha]_D$ - 1.0° : (c 0.8, $CHCl_3$)

λ_{max} : 253 nm (ε 11,400)

ν_{max} (neat) : 1650 (1,4-quinone) cm^{-1}

^{1}H NMR (80MHz) : δ 1.11 (3H,d,J=7Hz), 1.54 (3H,s), 1.64 (3H,d,J= Hz), 1.40 - 1.70 (2H,m), 1.90 (2H,bt,J=6Hz), 2.02 (3H,d,J=1.5Hz), 2.91 (1H,m), 5.02 (1H,m), 6.47 (1H,d, J=1Hz), 6.58 (1H,w,J=1.5Hz) ppm.

^{13}C NMR (20MHz) : δ 15.3(q), 17.7(q), 19.5(q), 25.7(q), 25.9(t), 31.4(d), 35.9(t), 123.9(d), 131.1(d), 132.0(s), 133.9(d), 145.1(s), 154.2(s), 187.4(s), 188.5(s) ppm.

Pseudopterogorgia blacquillensis

The air dried gorgonian (50g) was extracted with acetone (1L) and the solvent evaporated. The crude extract (6.0g) was dissolved in 10% aqueous methanol (150 mL) and extracted with light petroleum (3 x 75 mL). The aqueous methanol layer was diluted with water (75 mL) and extracted with dichloromethane (3 x 100 mL) to give a dark brown oil (3.7g). The dichloromethane extract was chromatographed on a short column using light petroleum with increasing quantities of ethyl acetate as eluting solvent. Elution with 5% ethyl acetate gave a fraction (1.0g) which was further purified by PLC to give (X) as a colourless oil (200 mg, 0.4%).

12-Hydroxy-*E*-γ-bisabolene (X)

ν_{max} (neat) : 3350 - 3450 (hydroxyl), 1670, 1450, 1380 cm^{-1}

^{1}H NMR (80MHz) : δ 1.67 (9H,bs), 1.95 (2H,m), 2.07 (2H, bs), 2.12 (2H,bs), 2.30 (2H,bt,J=6.5Hz), 2.70 (2H,bs), 3.96 (2H,bs), 5.30 (1H,m), 5.37 (1H,m) ppm.

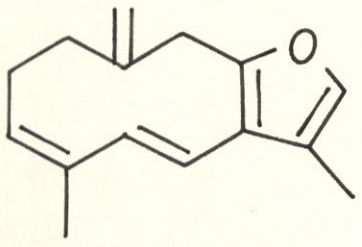
^{13}C NMR (20MHz) : δ 13.5(q), 18.3(q), 23.3(q), 26.6(t), 26.9(t), 29.7(t), 31.8(t), 33.7(t), 69.0(t), 120.7(d), 125.5(s), 126.2(d), 128.7(s), 134.1(s), 134.8(s) ppm.

12-Acetoxy-*E*-γ-bisabolene (XI)

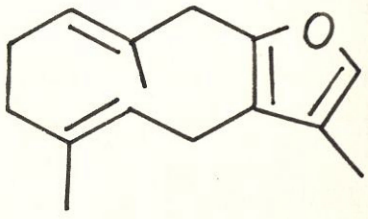
Alcohol (X) (40 mg), was dissolved in pyridine (1 mL) and acetic anhydride added (1 mL). After two hours the reaction was quenched with methanol (5 mL) and diluted with water (10 mL). The suspension was extracted with ethyl acetate (2 x 10 mL). The ethyl acetate extract was washed with dilute hydrochloric acid (2 x 10 mL) followed by water (3 x 10 mL). The organic phase was dried (Na_2SO_4) and the solvent evaporated. The crude acetate was purified by PLC with light petroleum-acetone (4:1) as the mobile phase, to give (XI) as a colourless oil (40 mg).

ν_{max} (neat) : 1720 (acetate), 1670, 1510, 1210 cm^{-1}

^1H NMR (80MHz) : δ 1.68 (9H,s), 1.95 (2H,m), 2.09 (3H,s), 2.32 (2H,bt,J=6Hz), 2.72 (2H,bs), 4.45 2H,bs), 5.35 (1H,m), 5.45 (1H,m) ppm.



(XV)



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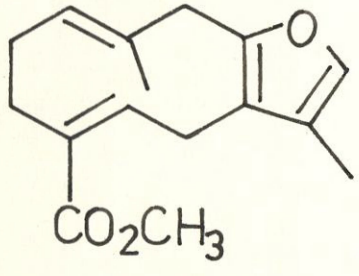
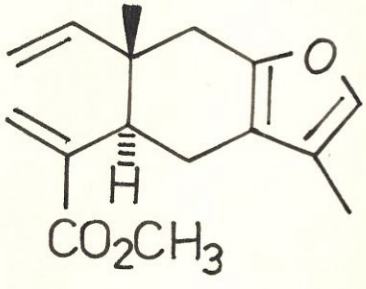


(XXVIII)



(XXIX)

CHAPTER THREE

AN INVESTIGATION INTO THE CONSTITUENTS OF *PSEUDOPTEROGORGIA* SP.

3.1 INTRODUCTION

Germacrane sesquiterpenes are widely distributed[9] within the orders Gorgonaceae, Alcyonaceae, and Stolonifera (subclass Octocorallia, Phylum Cnideria). In a recent investigation of the gorgonian *Pseudopterogorgia americana*, Fenical and his co-workers[9] isolated the germacrane derivative, furanotriene (XV). As part of a programme to isolate novel secondary metabolites from marine organisms, we investigated the chemical constituents from an unknown gorgonian belonging to the genus *Pseudopterogorgia*. In addition to the known germacrane derivatives furanodiene (XXVII) and neosericenine (XXVIII), and the elemane derivative isosericenine (XXIX), a new sesquiterpene (XXX), which is the anhydro-product of sericealactone (XXXI), were isolated.

3.2 EXTRACTION

Air dried *Pseudopterogorgia* sp. was extracted with dichloromethane and the solvent evaporated to give a viscous brown oil in 16.8% yield.

The crude extract was subjected to repeated short column chromatography. Elution with light petroleum gave a fraction which was purified by PLC, to give furanodiene (XXVII) as a colourless unstable oil, in about 0.23% of the crude extract. Elution with dichloromethane gave a fraction which consisted of an approximately 2:3 mixture of neosericenine (XXVIII), and isosericenine (XXIX). The mixture of (XXVIII) and (XXIX) were separated by silica HPLC with 2% ethyl acetate in isooctane as mobile phase. Further elution of the short column with dichloromethane gave a fraction which was purified by repeated PLC to give (XXX) as an oil.

3.3 GERMACRANE AND ELEMANE DERIVATIVES

Furanodiene (XXVII) was isolated as a colourless, unstable oil. The UV spectrum of (XXVII) had an absorption maximum at 220 nm (ε 10,000). The IR spectrum had absorption bands at 1560 and 850 cm^{-1} due to a substituted furan and a trisubstituted double bond respectively.

The ^{1}H NMR spectrum of (XXVII) showed resonances at δ 1.28 (3H,d,J=1Hz) and 1.59 (3H,bs) due to two vinyl methyls, while a doublet at δ 1.91 (3H,J=1.5Hz) was ascribed to a β-furan methyl. The β-furan methyl at δ 1.91 was allylically coupled to a broad singlet at δ 7.05 (1H), attributed to an α-hydrogen on a furan ring. The vinyl methyl signals at δ 1.28 and 1.59 were coupled to olefinic protons at δ 4.96 (1H,m) and 4.76 (1H,t,J=8Hz) respectively. Two additional signals ascribed to two methylene groups appeared as a broad quadruplet in an AB system at δ 3.44 and 3.52 (J=17Hz) and as a broad doublet at δ 3.08 (2H).

The spectroscopic data presented above are in complete accord with literature data[13a,b] for furanodiene. Furanodiene is widely distributed in octocorals.[9] It was first isolated from *Curcuma zedoaria* (Zingiberaceae) by Japanese workers.[13a]

Neosericenine (XXVIII) was isolated as a moderately stable oil, $[\alpha]_D$ ±0°. The UV spectrum of (XXVIII) had an absorption maximum at 210 nm (ε 11,000). The IR spectrum of (XXVIII) had absorption bands at 1713 (conjugated ester), 1628 (olefinic), 1560 (furan), 1242, 1209 and 765 cm^{-1}.

The ^{1}H NMR spectrum of (XXVIII) was similar to that of furanodiene (XXVII) except that in (XXVIII) one of the vinyl methyls was absent; in addition there was a new signal at δ 3.72 (3H,s) due to a methyl ester. The remaining vinyl methyl had a resonance at δ 1.20 (3H,d,J=1.5Hz) that was coupled to an olefinic proton at δ 5.07 (1H,t, J=7.5Hz). The presence of the substituted furan in (XXVIII) as in (XXVII) was evident from an aromatic methyl at δ 1.92 (3H,d,J=1.3Hz) that was coupled to an α-furan proton at δ 7.07 (1H,bs). A proton on a trisubstituted double bond resonated at δ 5.53 (1H,dd,J=10.6,3.3Hz).

The spectral data presented above are in agreement with literature data[14] for neosericenine. Neosericenine (XXVIII) was first isolated from the leaves of *Neolitsea sericea* Koidz (Lauraceae) by Takeda and his co-workers.[14] It was subsequently isolated from the Pacific gorgonians *Muricea austera* and *M. fungifera* by Fenical and his co-workers.[9]

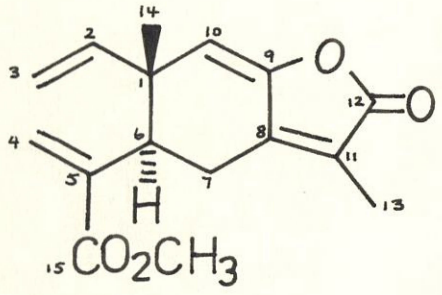
Isosericenine (XXIX) was isolated as an oil. The UV spectrum of (XXIX) had an absorption maximum at 209 nm (ε 16,000). The IR spectrum of (XXIX) had absorption bands at 1718 (conjugated ester), 1551, 1508 (furan), 1245 and 1133 cm^{-1}.

The ^{1}H NMR spectrum of (XXIX) had signals at δ 1.89 (3H,d,J=1Hz) and 7.00 (1H,bs) due to a substituted furan as in (XXVII) and (XXVIII). The presence of an ABX system in (XXIX) was evident from signals at δ 4.87 (1H,A), 4.89 (1H,B), and 5.78 (1H,X), J_{AB} = 1.7Hz, J_{AX} = 10.0Hz and J_{BX} = 17.0HZ. Two olefinic protons at δ 5.53 (1H,m) and 6.26 (1H,d,J=1.2Hz) were assigned to a terminal methylene that was conjugated to a methoxycarbonyl group. A resonance at δ 3.68 (3H,s) was readily assigned to a methyl ester. A methyl attached to a quarternary carbon showed a signal at δ 0.96 (3H,s). Signals for two methylene groups resonated between δ 2.00 and 2.50 while a methine proton resonated at δ 3.22 (1H,m).

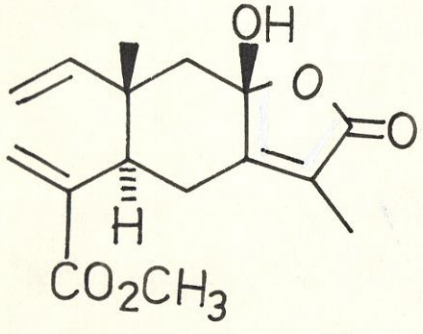
The above spectral data are in complete accord with literature data for isosericenine (XXIX), particularly the ^{1}H NMR spectrum which is identical to a published spectrum for (XXIX).[15]

Isosericenine (XXIX) was first isolated from leaves of *Neolitsea sericea* Koidz (Lauraceae),[15] and subsequently from the Pacific gorgonians *Muricea austera* and *M. fungifera*.[9]

Anhydrosericealactone (XXX) was isolated as a yellow gum, $[\alpha]_D$ +19° (c 0.05, $CHCl_3$). The molecular formula $C_{16}H_{18}O_4$, was revealed by a combination of low



(XXX)



(XXXI)

resolution mass spectrometry (M+, 274) and ^{13}C NMR spectroscopy. The UV spectrum of (XXX) had absorption maxima at 215 and 276 nm (ε 10,000, 12,000). The IR spectrum of (XXX) had absorption bands at 1765 (conjugated γ-lactone), 1720 (conjugated ester), 1670, 1655, and 1625 cm^{-1} (olefinic).

The ^{1}H NMR spectrum of (XXX) had signals for an ABX system as in (XXIX) at δ 4.99 (1H,dd,J=18,1.5Hz), 5.17 (1H,dd,J=10,1.5Hz) and 5.85 (1H,dd,J=18,10Hz). Two olefinic protons with resonances at δ 5.59 (1H,bs) and 6.34 (1H,s) were attributed to a terminal methylene that was conjugated to a methoxycarbonyl group at δ 3.75 (3H,s) as in (XXIX). The absence of an α-furan proton in (XXX) suggested that the furan ring was oxidized to a γ-lactone. A signal at δ 1.91 (3H,d,J=1Hz) was attributed to a methyl in the α-position of an α,β-unsaturated γ-lactone. Irradiation of the signal at δ 1.91 caused an olefinic proton at δ 5.50 (1H,bs) to sharpen while a broad doublet at δ 2.74 (2H) also sharpened. The broad doublet at δ 2.74 was further coupled to a signal at δ 3.32 (1H,t,J=8Hz). The ^{1}H NMR spectrum of (XXX) contained one more signal, which resonated at δ 1.12 (3H,s) and was assigned to a methyl group on a quarternary carbon. The spectral data suggested that (XXX) was the anhydro product of sericea lactone (XXXI), also isolated from *Neolitsea sericea*.[16]

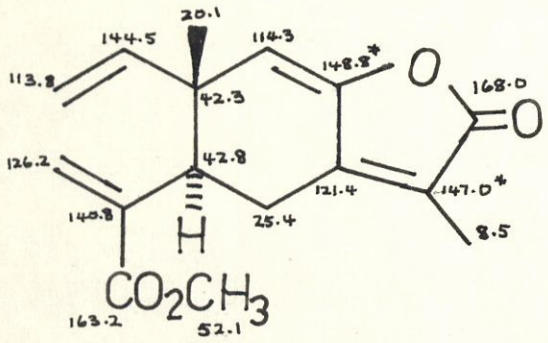
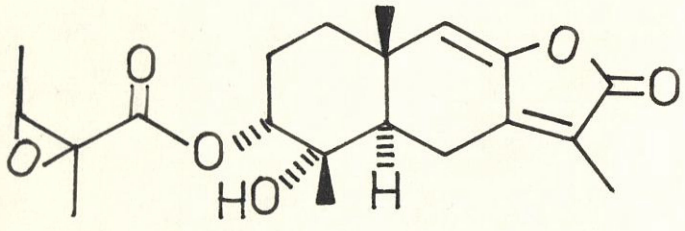


FIGURE I

* Signals that may be reversed.



(XXXII)

The ^{13}C NMR spectrum of (XXX) was in agreement with the proposed structure. A resonance at δ 8.5(q) was assigned to the α-methyl on the γ-lactone. A second methyl resonated at δ 20.1(q) while a methyl ester gave a signal at δ 52.1(q). Resonances at δ 144.5(d) and 113.8(t) were assigned to C-2 and C-3, while signals at δ 126.2(t) and 140.8(s) were assigned to C-4 and C-5. The remaining resonances were assigned as indicated in Figure I.

The conjugated α,β-unsaturated-γ-lactone functionality in (XXX) also occurs in the eudesmanolide (XXXII), isolated from *Pluchea rosea* (Compositae) by Bohlmann and his co-workers.[17]

3.4 EXPERIMENTAL

Air dried specimens of *Pseudopterogorgia* sp. (270g) were extracted with dichloromethane (2L) and the solvent evaporated to give a viscous brown oil (45g). The crude extract was subjected to short column chromatography and eluted with various solvents as indicated in Table 1.

TABLE 1

Fraction	Solvent System	Ratio	Volume/mL	Weight/g
1	Petroleum ether		1000	14.3
2	Petroleum-ether:CH_2Cl_2	9:1	300	12.1
3	Methylene chloride		200	4.3
4	CH_2Cl_2:Ethyl acetate	3:1	400	2.0

Fraction 1 (4.9) was further chromatographed on a short column and eluted with light petroleum to give a fraction (150 mg) which was purified by PLC to give furanodiene (20 mg) as an oil.

Furanodiene (XXVII)

λ_{max} : 220 nm (ε 11,000)

ν_{max} (neat) : 1560, 1140, 1080, 1028 and 850 cm^{-1}

^1H NMR (80MHz) : δ 1.28 (3H,d,J=1Hz), 1.59 (3H,bs), 1.91 (3H,d,J=1.5Hz), 3.08 (2H,bd,J=7.5Hz), 3.44, 3.25 (ABq,J=17Hz), 4.76 (1H,bt, J=7Hz), 4.96 (1H,m) and 7.05 (1H,bs).

Fraction 2 contained a mixture of unstable sesquiterpenes and was not investigated further.

Fraction 3 (1.0g) was subjected to silica HPLC with 2% ethyl acetate in isooctane to give neosericenine (214 mg) and isosericenine (321 mg).

Neosericenine (XXVIII)

(XXVIII) was obtained as a colourless oil

λ_{max} : 210 nm (ε 11,000)

ν_{max} (neat) : 1713, 1628, 1560, 1242, 1209 and 765 cm^{-1}

^1H NMR (80MHz) : δ 1.20 (3H,d,J=1.5Hz), 1.92 (3H,d, J=1.3Hz), 3.72 (3H,s), 5.07 (1H,t, J=7.5Hz), 5.53 (1H,dd,J=10.6,3.3Hz) and 7.07 (1H,bs) ppm.

Isosericenine (XXIX)

(XXIX) was obtained as a colourless oil

λ_{max} : 209 nm (ε 16,000)

ν_{max} (neat) : 1718, 1551, 1508, 1245 and 1133 cm^{-1}

^1H NMR (80MHz) : δ 0.96 (3H,s), 1.89 (3H,d,J=1Hz), 2.00 - 2.50 (4H,m), 3.22 (1H,m), 3.68 (3H,s), 4.87 (1H,dd,J=10.1.7Hz), 4.89 (1H,dd, J=17.5,1.7Hz), 5.53 (1H,m), 5.78 (1H,dd, J=17.5,10Hz), 6.26 (1H,d,J=1.2Hz) and 7.00 (1H,bs) ppm.

Fraction 4 (2.0g) was subjected to short column chromatography, eluting with light petroleum containing increasing concentrations of dichloromethane. Elution with pure dichloromethane gave a fraction (50 mg) that was purified by PLC [light petroleum-acetone (4:1)] to give anhydrosericealactone (59 mg) as a moderately stable gum.

Anhydrosericealactone (XXX)

(XXX) was obtained as a yellow gum

$[\alpha]_D$ + 19° : (c 0.05, $CHCl_3$)

λ_{max} : 215 nm (ε 10,000) and 276 nm (ε 12,000)

ν_{max} ($CHCl_3$) : 3080, 3020, 1765, 1720, 1670, 1655, and 1625 cm^{-1}

^1H NMR (80MHz) : δ 1.12 (3H,s), 1.91 (3H,d,J=1Hz), 2.74 (2H,bd,J=8Hz), 3.22 (1H,t,J=8Hz), 3.75 (3H,s), 4.99 (1H,dd,J=18,1.5Hz), 5.17 (1H,dd,J=10,1.5Hz), 5.50 (1H,bs), 5.59 (1H,bs), 5.85 (1H,dd,J=10,10Hz) and 6.34 (1H,s) ppm.

^{13}C NMR (20MHz) : δ 8.5(q), 20.1(q), 25.4(t), 42.3(s), 42.8(d), 52.1(q), 113.8(t), 114.3(d), 121.4(s), 126.2(t), 140.8(s), 144.5(d), 147.0(s), 148.8(s), 163.2(s) and 168.0(s) ppm.

Mass spectrum : m/z 274 (M+, $C_{16}H_{18}O_4$, 8%), 246(100), 231(12), 214(19), 186(22), 171(14), 159(33), 143(15), 132(18), 129(16), 122(20), 91(26).

CHAPTER FOUR

REINVESTIGATION OF THE CONSTITUENTS OF *PSEUDOPTEROGORGIA AMERICANA* (GMELIN)

4.1 INTRODUCTION

The gorgonian coral, *Pseudopterogorgia americana* (Gmelin), has been the subject of two previous investigations. Weinheimer and his co-workers reported the occurrence of 9-aristolene(I), 1(10)-aristolene(II), (+)-γ-maaliene(III), and (+)-β-gorgonene(IV) in *P. americana*.[5] More recently Fenical's group isolated furanotriene (XV) as a moderately stable oil from *P. americana*.[9] The structure of (XV) was arrived at after extensive spin-decoupling experiments and a study of molecular models. In their report, Fenical and his group indicated that complex mixtures of mainly unpurifiable materials were obtained on chloroform extraction of *P. americana*. It was therefore decided to undertake a reinvestigation of *P. americana*.

4.2 EXTRACTION

Partially dried *Pseudopterogorgia americana* was extracted with cold acetone. The acetone extract was partitioned between light petroleum and 10% aqueous methanol. Evaporation of the light petroleum extract in vacuo yielded a dark green oil (6.2%).

The oil was subjected to careful PLC using light petroleum as the developing solvent (three developments). Four germacrane derivatives and one furanoguaiane were isolated, after visualization of the chromatoplates at short wavelength UV (254 nm). These were designated as compounds A, B, C, D, and E, in order of decreasing R_f values.

4.3 THE STRUCTURE OF GERMACRANE DERIVATIVES A, B, C, AND D, AND FURANOGUAIANE DERIVATIVE E

STRUCTURE OF COMPOUND A

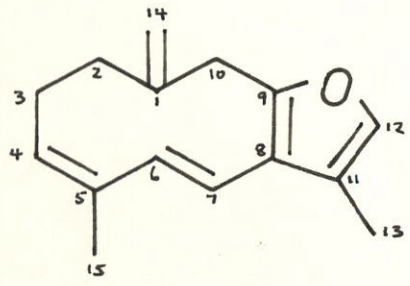
Compound A was isolated as a colourless oil, $[\alpha]_D$ + 238° (c 0.31, $CHCl_3$). The molecular formula, $C_{15}H_{20}$ (M+, 204) was revealed by a combination of low resolution mass spectrometry and ^{13}C NMR spectroscopy.

The UV spectrum of compound A had an absorption maximum at 258 nm (ε 4500) which indicated the presence of a conjugated double bond. This was supported by data from

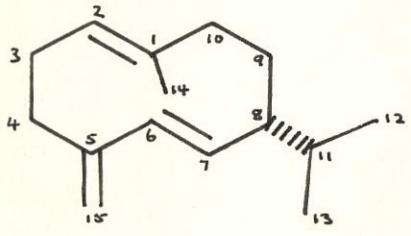
the IR spectrum, which had a band at 1630 cm^{-1}.[18] IR bands at 980 and 880 cm^{-1} indicated the presence of *trans*-disubstituted and terminal olefinic functions respectively. Two approximately equal absorptions at 1385 and 1370 cm^{-1} revealed the presence of an isopropyl group in compound A.

The ^{1}H NMR spectrum of compound A confirmed the presence of an isopropyl moiety; it had doublets at δ 0.84 (3H,J=6Hz) and at δ 0.88 (3H,J=6Hz). A methyl group attached to a double bond was revealed by a signal at δ 1.55 (3H,bs). The olefinic region of the ^{1}H NMR spectrum of compound A had signals for five protons. Signals at δ 4.75 (1H,d,J=3Hz) and 4.80 (1H,dd,J=3,1Hz) were attributed to a terminal methylene group, while a multiplet at δ 5.00 - 5.05 (1H) was assigned to a proton attached to a trisubstituted double bond. The AB part of an ABX spin system was revealed by signals at δ 5.24 (A) and 5.83 (J_{AB}=16Hz, J_{AX}=6Hz).

The ^{13}C NMR spectrum of compound A was in complete accord with the ^{1}H NMR assignments. Thus, signals at δ 16.5(q) and 19.9(q) were assigned to isopropyl methyl groups, while a resonance at δ 21.3(q) was attributed to a methyl group attached to a double bond. The low field region of the ^{13}C NMR spectrum had signals for six sp^2 carbons. Signals at δ 109.5(t) and 148.7(s) were attributed



(XV)



(XXXIII)

to an exocyclic methylene group. The resonances at δ 134.1(d) and 136.1(d) were assigned to a *trans*-disubstituted double bond, while a signal at δ 130.3(d), and 134.5(s) were attributed to a trisubstituted double bond. Since compound A, $C_{15}H_{24}$, contain four double bond equivalents, three of which are accounted for, the compound must be monocyclic.

Germacrane derivatives are widely distributed in octocorals.[9] A review of the literature indicated that compound A had spectroscopic properties (UV, IR, ^{1}H NMR, MS) identical to germacrene-D, except for the optical rotation, $[\alpha]_D$ + 238° (lit.,[19] $[\alpha]_D$ - 240°). The above evidence suggest that compound A is the antipode of (-)-germacrene-D (XXXIII), isolated from terrestrial sources.[19,20] The isolation of compounds from marine organisms that are antipodal to their terrestrial counterparts has precedence.[21] This appears to be the first reported occurrence of (+)-germacrene-D from a natural source.

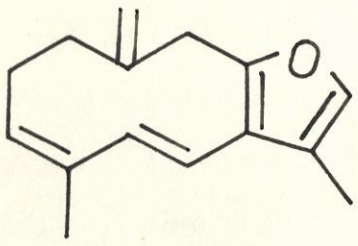
STRUCTURE OF COMPOUND B

The molecular formula, $C_{15}H_{18}O$ (M+, 214) was established for compound B by a combination of low resolution mass spectrometry, and ^{13}C NMR spectroscopy. The UV spectrum had absorptions at 225 nm (ε 11,300) and 265 (ε 1100),

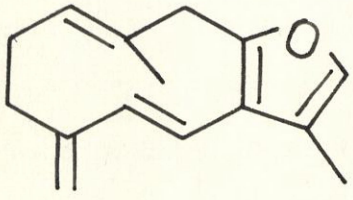
assignable to a substituted furan, and a conjugated double bond, respectively.

The IR spectrum of compound B showed bands for *gem*-disubstituted (3080, 1640, 890 cm^{-1}), *trans*-disubstituted (970 cm^{-1}), and trisubstituted (840 cm^{-1}) double bonds. The presence of a substituted furan in compound B was revealed by IR bands at 1550, and 750 cm^{-1}.

The ^{1}H NMR spectrum of compound B had six low field signals. A resonance at δ 7.03 (1H,q,J=1Hz) which was coupled to an aromatic methyl group δ 1.88 (3H,d,J=1Hz), was attributed to an α-proton on an α,β,β'-methyl-tri-substituted furan. Signals for the AB part of an ABX system resonated at δ 5.99 (1H,dd,J=12,1Hz) and 5.86 (1H,d,J=12Hz). Irradiation of the signal at δ 5.99 caused a doublet of doublets at δ 4.55 (1H,J=2.5,1Hz) to collapse into a doublet. The signal at δ 4.55 was further coupled to a doublet at δ 4.71 (1H,J=2.5Hz); both signals were attributed to the protons on a *gem*-disubstituted double bond. This was confirmed by further decoupling experiments. The remaining low field resonance, which occurred at δ 5.25 (1H,bt, J=7Hz), was readily assigned to a proton on a trisubstituted double bond. An olefinic methyl group had a doublet at δ 1.50 (3H,J=1.5Hz); it was allylically coupled to the



(XV)



(XXXIV)

olefinic proton at δ 5.25 ppm. Two high field signals occurred at δ 2.18 (4H,m), and 3.21 (2H,bs).

The above assignments for compound B were fully supported by the ^{13}C NMR spectrum (Table 2). A high field signal at δ 8.8(q) was assigned to a β-methyl substituted furan. Resonances at δ 118.2(s), 123.3(s), 139.0(d), and 144.8(s) were readily assigned to the furan ring. An exocyclic methylene group was evident from signals at δ 113.6(t) and 148.4(s), while signals at δ 119.9(d) and 125.5(d) indicated the presence of a disubstituted double bond. There were four remaining ^{13}C NMR signals at δ 17.0(q), 25.8(t), 32.2(t), and 39.4(t), due to a methyl and three methylene groups, respectively. The above assignments accounted for six of the seven double bond equivalents of compound B. Compound B is therefore, monocarbocyclic.

A comparison of the ^{1}H, and ^{13}C NMR data of compound B with the published data for furanotriene (XV) strongly suggested that they were identical. However, the coupling observed between one of the protons [δ 5.99 (1H, dd,J=12,1Hz)] of the *trans*-disubstituted double bond, and one of the protons [δ 4.55 (1H,dd,J=2.5,1Hz)] of the exocyclic methylene group indicated that the structure (XV) for furanotriene should be revised to (XXXIV). In their

TABLE 2

^{13}C NMR data for compounds A - C*

C#	A	B	C
1	134.5	134.2	148.4[a]
2	130.3	136.2	38.6[b]
3	27.1[a]	25.8	29.3[b]
4	41.3	32.3	132.2
5	149.5	148.3	136.4
6	134.1	119.9	113.6
7	136.1	125.5	127.1
8	53.6	118.2	120.4
9	29.9[a]	144.8	150.2[a]
10	35.1	39.5	40.3
11	33.4	123.3	121.4
12	16.5	139.0	139.5
13	19.9	8.7	8.3
14	21.3	17.0	107.9
15	109.6	113.6	16.7

* Recorded at 20MHz in $CDCl_3$ solution. Assignments are based upon J-Modulated Spin Echo experiments, and comparison with literature data where applicable.

a,b Indicates assignments within a column that may be reversed.

report, Fenical and his group[9] indicated that furanotriene had an unexpected UV absorption at 219 nm. This was taken to mean that the *P*-orbitals of the furan and the tri-substituted double bond were orthogonal to those of the disubstituted double bond, eliminating conjugation between these respective chromophores. In our hands, however, furanotriene had UV absorptions at 225 and 265 nm. This is an indication that conjugation in fact exists in the diene system of furanotriene.

STRUCTURE OF COMPOUND C

Compound C, $C_{15}H_{18}O$ (M+, 214), was isomeric with furanotriene (XXXIV). UV absorptions at 215 nm (ε 8200), and 260 nm (ε 3500) revealed the presence of a substituted furan and a conjugated system in compound C.

The IR spectrum of compound C showed absorption bands at 3080, 890 (exocyclic methylene), 1625 (conjugated double bond), and 1550 (furan) cm^{-1}.

The ^1H NMR spectrum of compound C was similar to that of furanotriene (XXXIV). Signals for a *trans*-disubstituted double bond occurred as an AB system at δ 5.99 (1H, d,J=15Hz), and 5.91 (1H,d,J=15Hz). An exocyclic methylene group was evident from resonances at δ 5.05 (1H,d,J=2Hz), and 4.75 (1H,bs). The proton which resonated at δ 4.75 was

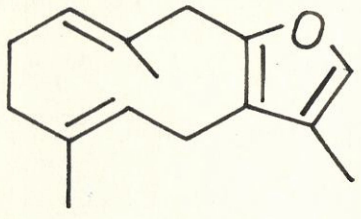
coupled to a doubly allylic methylene group at δ 3.34 (1H, d,J=16Hz) and 3.55 (1H,d,J=16Hz). An aromatic and a vinyl methyl group had signals at δ 1.90 (3H,d,J=1.5Hz) and 1.30 (3H,s), respectively. The aromatic methyl group was coupled to a proton at δ 7.05 (1H,bs). An olefinic proton which resonated at δ 5.47 (1H,bt,J=7Hz) was assigned to a trisubstituted double bond.

The ^{13}C NMR spectrum (Table 2) of compound C was also similar to that of (XXXIV). The above data can readily be accommodated by structure (XV), previously assigned to furanotriene. The name isofuranotriene (XV) is proposed for this new compound.

STRUCTURE OF COMPOUND D

Compound D was isolated as an optically inactive, colourless oil. The molecular formula, $C_{15}H_{20}O$ (M+, 216), indicated six degrees of unsaturation in compound D. A UV absorption at 219 nm (ε 5000), suggested that compound D possessed a substituted furan. The IR spectrum of compound D had absorption bands at 1560 (furan), and 850 (trisubstituted double bond) cm^{-1}.

The ^{1}H NMR spectrum of compound D had signals for one aromatic and two vinyl methyl groups. The aromatic methyl group resonated at δ 1.91 (3H,d,J=1.2Hz), while the

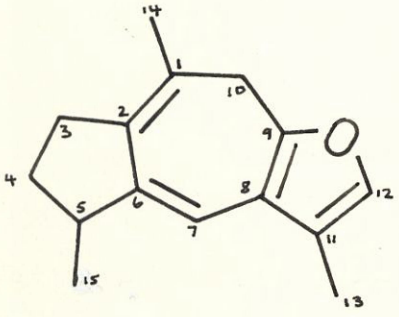


(XXVII)

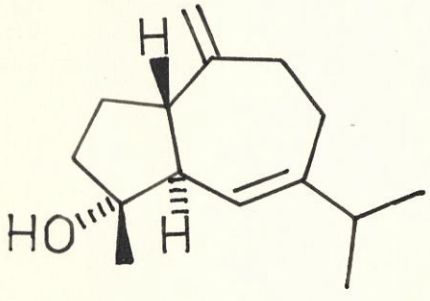
two vinyl methyls occurred at δ 1.59 (3H,bs) and 1.28 (3H,d,J=1Hz), respectively. Signals for two olefinic protons attached to trisubstituted double bonds were evident at δ 4.76 (1H,bt,J=8Hz) and 4.96 (1H,bt,J=7Hz). A signal at δ 7.05 (1H,bs) was readily assigned to an α-furan proton. Comparison of the above data with the data reported for furanodiene (XXVII)[13a,b] revealed that both compounds were identical. Furanodiene (XXVII) was first isolated from *Curcuma zedoaria* (Zingiberaceae); it appears to be ubiquitous in the marine environment.[9] The possibility that furanodiene is a common precursor for furanotriene (XXXIV) and isofuranotriene (XV) seems very attractive.

STRUCTURE OF COMPOUND E

Compound E, a yellow, unstable oil, had $[\alpha]_D$ + 72.4° (c 0.29, $CHCl_3$). The molecular formula, $C_{15}H_{18}O$ (M+, 214), indicated seven degrees of unsaturation. The UV spectrum had absorptions at 219 nm (ε 9000) and 250 nm (ε 2900); this suggested the presence of a substituted furan and a conjugated system respectively, in compound E. The IR spectrum of compound E had absorption bands at 1640 (double bond) and 1560 (furan) cm^{-1}.



(XXXV)



(XXXVI)

The ^{1}H NMR spectrum of compound E had resonances for three methyl groups at δ 1.34 (3H,d,J=6.5Hz) and 2.00 (6H,bs). Low field signals at δ 5.90 (1H,bs) and 7.05 (1H,bs) were attributed to an olefinic proton on a trisubstituted double bond, and an α-furan proton, respectively. Irradiation of the signal at δ 2.00 caused the signal at δ 7.05 to sharpen while the broad singlet at δ 5.90 collapsed into a doublet (J=1.5Hz). A bis-allylic methylene group was observed as an AB system at δ 3.26 and 3.38 (J_{AB}=12Hz). Resonances for five remaining protons occurred between δ 2.00 and 3.10 (5H,m). The furan ring, along with two double bonds accounted for, indicated that compound E was tricyclic.

Due to the unstable nature of compound E, attempts to record its ^{13}C NMR spectrum were unsuccessful. However, the spectroscopic data presented above, along with decoupling experiments, suggested that compound E had a furanoguaiane skeleton. Structure (XXXV) is proposed for compound E. The stereochemistry at C-5 remains undefined.

This appears to be the first reported occurrence of a furanoguaiane in the marine environment. However, the possibility that (XXXV) might be an artifact derived from a germacrane derivative cannot be excluded. Recently,[22] the guaiane (XXXVI) was isolated from the Australian soft coral *Nephthea chabrallii*.

4.4 EXPERIMENTAL

EXTRACTION

Partly dried specimens of *Pseudopterogorgia americana* (400g) were minced, and allowed to stand in acetone (1.5L) for 24 hours. The acetone extract was filtered and concentrated in vacuo to an aqueous suspension (50mL). The aqueous suspension was partitioned between light petroleum (3 x 200 mL) and 10% aqueous methanol (500 mL). The light petroleum extract, after evaporation of the solvent, gave a dark green oil (24.9g, 6.2%0).

ISOLATION

The extract (1.0g), was subjected to careful PLC with light petroleum as mobile phase (two developments). The chromatoplates were observed at short wavelength (254 nm) UV radiation, and seven bands recovered to give fractions 1 - 7 (Table 3).

TABLE 3

PLC fractions of extract (1.0g) of *P. americana*

Fraction	Colour (254 nm)	Weight/g	Content
1	purple	0.032	compound E
2	purple	0.085	compounds C and D
3	purple	0.046	compound B
4	yellow	0.020	unindentified sesquiterpene
5	purple	0.069	compound A
6	purple	0.044	sesquiterpene hydrocarbons
7	yellow	0.128	sesquiterpene hydrocarbons

Fraction 2 (85 mg) was further chromatographed (PLC) using silver nitrate (15%)-impregnated silica, and light petroleum-ethyl acetate (90:10) as eluting solvents, to give, in order of increasing R_fs, compound C (30 mg) and compound D (15 mg).

COMPOUND A (XXXIII)

(XXXIII) was obtained as a colourless oil

$[\alpha]_D^{22}$ + 238° : (c 0.32, $CHCl_3$) [lit. $[\alpha]_D$ - 240°]

λ_{max} : 258 nm (ε 4500)

ν_{max} (neat) : 3080, 3020, 1630, 1385, 1370, 980, 970 and 880 cm^{-1}

^{1}H NMR (80MHz) : δ 0.84 (3H,d,J=6Hz), 0.88 (3H,d,J=6Hz), 1.55 (3H,bs), 1.50 - 2.50 (8H,m), 4.75 (1H,d,J=3Hz), 4.80 (1H,dd,J=3,1Hz), 5.00 - 5.05 (1H,m), 5.24 (1H,dd,J=16, 6Hz), 5.83 (1H,dd,J=16,1Hz) ppm.

^{13}C NMR (20MHz) : 16.5(q), 19.9(q), 21.3(q), 27.1(t), 29.9(t), 33.4(d), 35.1(t), 41.3(t), 53.6(d), 109.6(t), 130.3(d), 134.1(d), 134.5(s), 136.1(d), 149.5(s) ppm.

Mass spectrum : m/z 204 (M+, 17%), 161(100), 119(53), 107(18), 105(80), 91(66), 79(40), 77(32).

COMPOUND B (XXXIV)

(XXXIV) was obtained as a yellow, unstable oil

$[\alpha]_D^{22} \pm 0^o$: (c 0.365, $CHCl_3$)

λ_{max} : 225 nm (ε 11,300), 265 nm (ε 1100)

ν_{max} (neat) : 3080, 3020, 1640, 1630, 1550, 970, 890, 840, 750 and 740 cm^{-1}

^{1}H NMR (80MHz) : δ 1.50 (3H,d,J=1.5Hz), 1.88 (3H,d,J=1Hz), 2.18 (4H,m), 3.21 (2H,bs), 4.55 (1H,dd, J=2.5,1Hz), 4.71 (1H,d,J=2.5Hz), 5.25 (1H,t,J=7Hz), 5.86 (1H,d,J=12Hz), 5.99 (1H,dd,J=12,1Hz), 7.03 (1H,q,J=1Hz) ppm.

^{13}C NMR (20MHz) : 8.7(q), 17.0(q), 25.8(t), 32.3(t), 39.4(t), 113.6(t), 118.2(s), 119.8(d), 123.3(s), 134.2(s), 136.2(d), 139.0(d), 144.8(s) and 148.3(s) ppm.

Mass spectrum : m/z 214 (M+, 18%), 199(19), 158(89), 145(100), 115(83), 105(22), 91(96), 79(25), 77(64).

COMPOUND C (XV)

(XV) was obtained as a yellow unstable oil

$[\alpha]_D^{22} \pm 0^o$: (c 0.415, $CHCl_3$)

λ_{max} : 215 nm (ε 8200) and 260 nm (ε 3500)

ν_{max} (neat) : 3080, 1625, 1550 and 890 cm^{-1}

^1H NMR (80MHz) : 1.30 (3H,s), 1.90 (3H,d,J=1.5Hz), 2.02 - 2.50 (4H,m), 3.34, 3.55 (AB,J=16Hz), 4.75 (1H,bs), 5.05 (1H,d,J=2Hz), 5.47 (1H,t,J=7Hz), 5.91, 5.99 (AB,J=15Hz), 7.05 (1H,bs) ppm.

^{13}C NMR (20MHz) : 8.3(q), 16.7(q), 29.3(t), 38.6(t), 40.3(t), 107.9(t), 113.6(d), 120.4(s), 121.4(s), 127.1(d), 132.2(d), 136.4(s), 139.5(d), 148.4(s), 150.2(s) ppm.

Mass spectrum : m/z 214 (M+, 24%), 199(17), 158(89), 145(92), 115(78), 105(26), 91(100), 79(34), 77(68).

COMPOUND D (XXVII)

(XXVII) was obtained as a colourless oil

λ_{max} : 219 nm (ε 5000)

ν_{max} : 1560, 850 cm^{-1}

^1H NMR (80MHz) : δ 1.28 (3H,d,J=1Hz), 1.59 (3H,bs), 1.91 (3H,d,J=1.2Hz), 3.08 (1H,d,J=8Hz), 3.44, 3.52 (J_{AB}=17Hz), 4.76 (1H,bt,J=8Hz), 4.96 (1H,bt,J=7Hz), 7.05 (1H,bs) ppm.

Mass spectrum : m/z 216 (M+, $C_{15}H_{20}O$, 51%), 201(15), 159(33), 145(34), 115(18), 109(23), 108(100), 105(41), 91(44), 79(30), 77(33).

COMPOUND E (XXXV)

(XXXV) was obtained as an unstable yellow oil

$[\alpha]_D^{22}$ + 72.4° : (c 0.29, $CHCl_3$)

λ_{max} : 219 nm (ε 9000) and 250 nm (ε 2900)

ν_{max} : 1640, 1560 cm^{-1}

^{1}H NMR (80MHz) : 1.34 (3H,d,J=6.5Hz), 2.00 (6H,s), 2.00 - 3.10 (5H,m), 3.26, 3.38 (J_{AB}=12Hz), 5.90 (1H,bs), 7.05 (1H,bs) ppm.

Mass spectrum : m/z 214 (M+, $C_{15}H_{18}O$, 42%), 199(17), 157(17), 143(13), 115(23), 108(100), 105(11), 91(36), 79(26), 77(26).

CHAPTER FIVE

AN INVESTIGATION INTO THE CONSTITUTENTS OF *PSEUDOPTEROGORGIA ACEROSA* (PALLAS)

5.1 INTRODUCTION

Chemical investigation of *Pseudopterogorgia acerosa* (Pallas) by Fenical and his co-workers [10] led to the isolation of pseudopterolide (XVI), an irregular diterpenoid with cytotoxic properties. Investigation of *P. acerosa* collected locally, resulted in the isolation of two furanoditerpenoids, designated as compounds F and G.

5.2 EXTRACTION

Air dried *Pseudopterogorgia acerosa* was extracted with acetone for 24 hours. The resulting extract was filtered and concentrated to an aqueous suspension. The aqueous suspension was partitioned between 20% aqueous methanol and light petroleum. The aqueous methanol layer was diluted with water (40%) and extracted with chloroform. Removal of the chloroform in vacuo, yielded a dark red gum (7.3g, 3.65%).

The chloroform extract (4.5g) was subjected to short column chromatography, eluting with light petroleum-ethyl acetate mixtures. Elution with 75% ethyl acetate afforded an acidic fraction which showed one spot on TLC plates. Treatment of the acidic material with ethereal diazomethane gave a mixture which had three spots on TLC plates.

The resulting mixture of methyl esters were separated into three bands by PLC, with light petroleum-ethyl acetate (3:1) as developing solvent. The material recovered from the band of highest R_f value was recrystallized from light petroleum-acetone to give white prisms, m.p. 130 - 132^{o} (compound F). The second band proved to be a mixture of at least three compounds, and was not

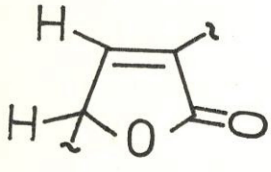
investigated further. The third band consisted of a single compound (compound G), but failed to crystallize in a number of solvents.

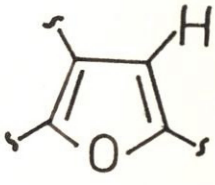
5.3 STRUCTURE OF COMPOUND F

Compound F was recrystallized from light petroleum-acetone as prisms, m.p. 130 - 132°, $[\alpha]_D$ + 62° (c 0.225, $CHCl_3$). The molecular formula, $C_{21}H_{24}O_5$, became evident from an ion at m/z 374 $(M + NH_4)^+$ in the chemical ionisation (NH_3) mass spectrum. The number of carbons and hydrogens corresponded in the ^{13}C and ^1H NMR spectra (see later). In passing, it should be noted that the high resolution electron impact mass spectrum (HR-EIMS) did not show the molecular ion - the peak at highest mass corresponded with $C_{14}H_{22}O$ (m/z obsd. 206.1670, calcd. for $C_{14}H_{22}O$: 206.1671).[24]

The UV spectrum had an absorption maximum at 245 nm (ε 6000). The infrared spectrum of compound F had no hydroxyl groups but indicated the presence of γ-lactone and ester (1760, 1720 cm^{-1} respectively) and terminal olefin (895 cm^{-1}).

The ^1H NMR (80MHz) spectrum showed that there were three methyl groups - a methyl ester (δ 3.83), as required by its genesis, together with two olefinic methyls resonating as broadened singlets at δ 1.79 and 1.99. Irradiation of the signal at δ 1.79 resulted in the collapse of resonances at δ 4.82 (1H,bs) and 5.05 (1H,bs) into doublets (J=2Hz) while irradiation of the signal at δ 1.99





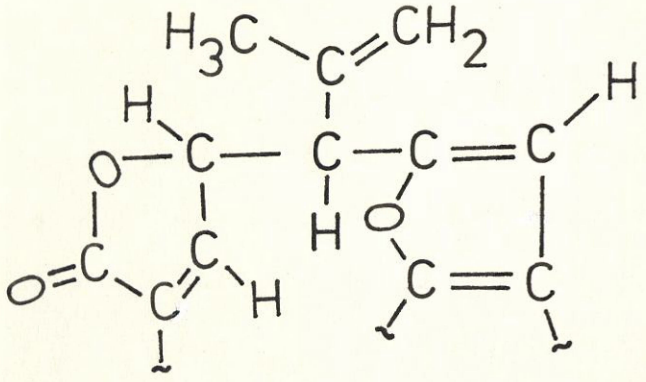
caused signals at δ 4.86 (1H,bs) and 5.07 (1H,bs) to sharpen. Thus, compound F has two isopropenyl groups.

The low field region of the spectrum had two other signals which could be reasonably assigned. One, a broadened singlet (1H) at δ 6.39 could be attributed to a β-proton in a 1,2,4-trisubstituted furan. The other, again a broadened singlet (1H) at δ 6.75 could be assigned to the β-proton on an α-substituted α,β-unsaturated γ-lactone.

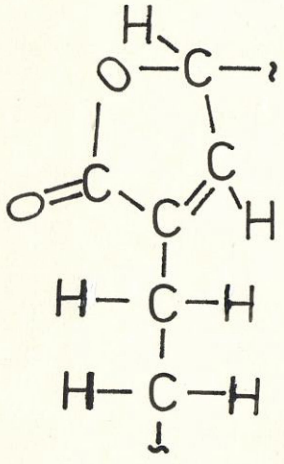
The functionalities suggested have accounted for all the oxygen atoms in compound F together with nine of the ten double bond equivalents required by the molecular formula. This indicated that compound F was monocarbocyclic.

The J-modulated spin echo ^{13}C NMR (100MHz) spectrum[24] showed signals for all twenty-one carbons. There were three methyl carbons (δ 18.7, 21.0, 51.0) and five methylene carbons, of which two were sp^2 hybridized (δ 21.5, 31.5, 37.5, 111.5, 115.5). In addition, there were five methine carbons (two sp^2 hybridized) at δ 41.5, 48.5, 80.7, 110.5 and 147.5, and eight quaternary sp^2 hybridized carbons (δ 116.0, 138.0, 142.0, 148.0, 151.2, 159.7, 165.0, 176.0).

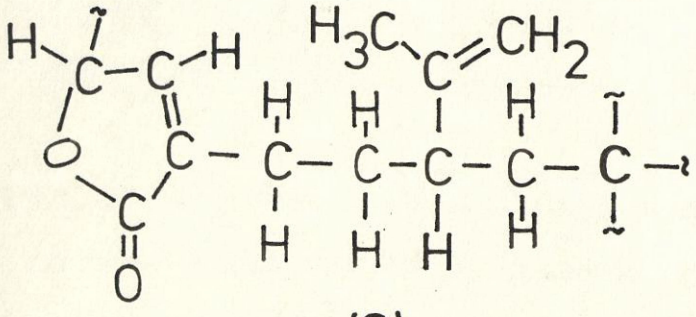
These signals could be assigned, in part, to the functional groups already indicated above by other physical measurements and reinforce their presence (see opposite page). The location and connectivities of three methylene



(A)



(B)



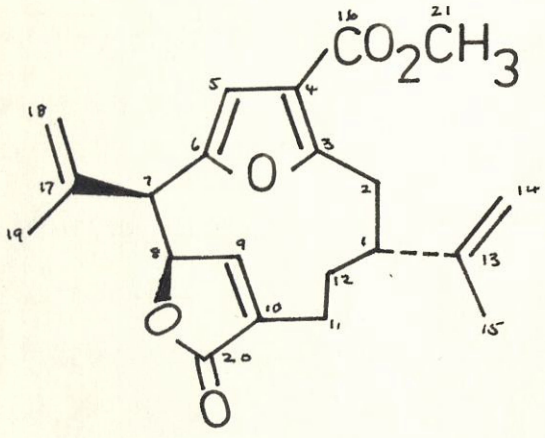
(C)

and two methine groups within a monocarbocyclic framework remained to be delineated. This was done by further decoupling experiments.

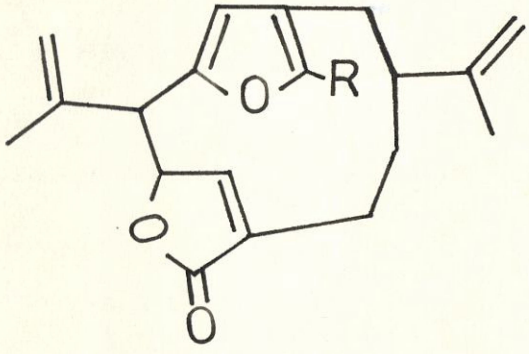
The part structure (A) was derived by decoupling experiments at 80MHz. Thus, irradiation of a one-proton signal at δ 3.84 (partly hidden by the methyl ester group) caused the broadened singlets at δ 4.86, 5.07 and 6.39 to sharpen while a doublet of doublets at δ 5.44 (1H,J=4.1Hz) collapsed into a broad singlet. Again, the resonance at δ 6.75 (1H,bs) sharpened when the signal at 5.44 was irradiated.

The connectivities of the three methylenes and one methine required decoupling experiments in the high field region of the spectrum. This was impractical at 80MHz and recourse had to be sought in spectra at higher field strengths.

Irradiation of a broad doublet at δ 2.21 (1H, J=14Hz) in the 360MHz ^{1}H NMR spectrum[24] of compound F, resulted in the collapse of a triplet of doublets at δ 0.84 (1H,J=14,14,3Hz) and 2.44 (1H,J=14,14,3Hz) while the broad singlet at δ 6.75 (1H) sharpened. On the other hand, irradiation of the signal at δ 0.84 led to the collapse of the signals at δ 2.21 and 2.44 into mutually coupled, broadened doublets (J=14Hz). In addition, a partly hidden signal at δ 1.80 (1H,bs) was affected. The large coupling between the resonances at δ 2.21 and 2.44 indicated that



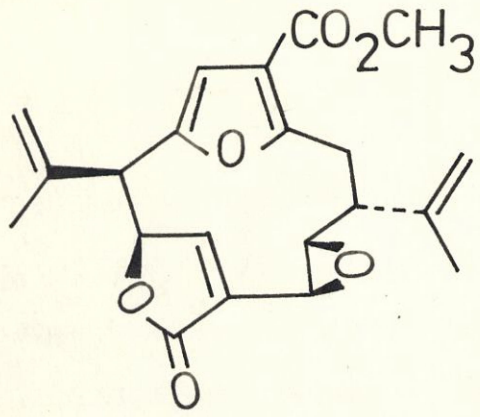
(XXXVI)



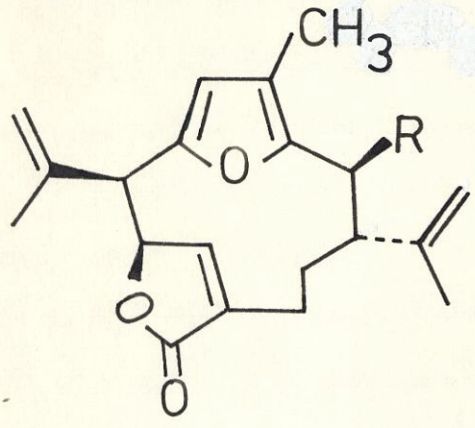
(XXXVII), $R = CO_2CH_3$

they were geminal. This was confirmed by irradiation of the triplet of doublets at δ 2.44, which led to the collapse of the broad doublet at δ 2.21, and also the signals at δ 0.84 and 1.80. This allowed extension of the partial structure (A) to partial structure (B).

When a resonance at δ 3.32 (1H,dd,J=16,12Hz) was irradiated, signals at δ 2.73 (1H,d,J=16Hz) and 2.78 (1H,m) collapsed into mutually coupled doublets ($J_{AB} \simeq$ 10Hz). The spin decoupling data at 360MHz indicated the part structure (C). Combined with data already given, this leads to two possible structures for compound F (see opposite page). Structure (XXXVI) was chosen on the basis of the fact that compounds with the same skeleton were previously isolated from gorgonians of the same genus. Thus, pseudopterolide (XVI) was isolated from *P. acerosa* while kallolide A - C (XX - XXII) and kallolide A acetate (XXIII) were isolated from *P. kallos*.



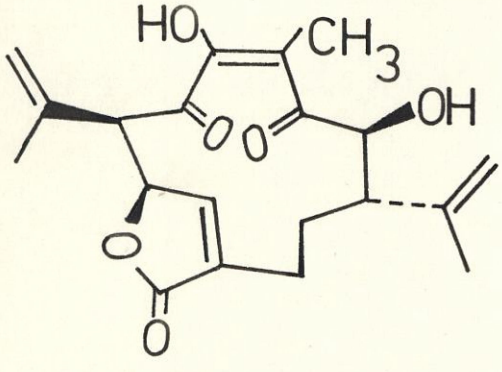
(XVI)



XX R = OH

XXI R = H

XXIII: R = OAc



(XXII)

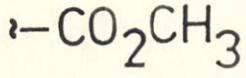
5.4 STRUCTURE OF COMPOUND G

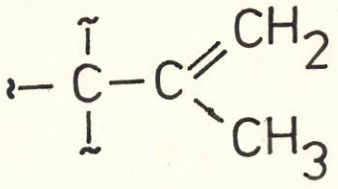
Compound G was isolated as a moderately unstable gum, $[\alpha]_D$ + 35° (c 0.425, $CHCl_3$). The molecular formula, $C_{21}H_{22}O_6$ was revealed by high resolution electron impact mass spectrometry (m/z obsd. 370.1447, calcd. for $C_{21}H_{22}O_6$: 370.1416).

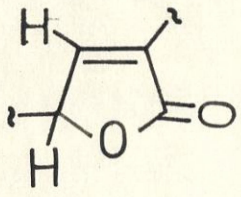
The UV spectrum had an absorption maximum at 253 nm (ε 6800) while the infrared spectrum showed bands indicative of the presence of γ-lactone (1760 cm^{-1}), conjugated ester (1715 cm^{-1}), saturated ketone (1710 cm^{-1}), and olefin (1640 cm^{-1}).

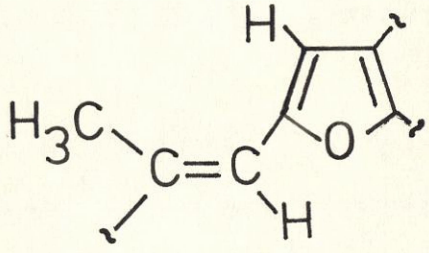
The ^1H NMR (80MHz) spectrum showed signals for three methyl groups - a methyl ester (δ 3.81), together with two olefinic methyls resonating at δ 1.56 (d,J=1.5Hz) and 1.79 (t,J=1Hz). The latter signal when irradiated, caused a broad signal at δ 5.08 (2H) to sharpen. This indicated the presence of an isopropenyl group in compound G. Similarly, irradiation of the olefinic methyl at δ 1.56 led to the collapse of a broadened singlet at δ 5.93 (1H) into a broad doublet (J=2Hz). A trisubstituted double bond is revealed, instead of a second isopropenyl group as in compound F.

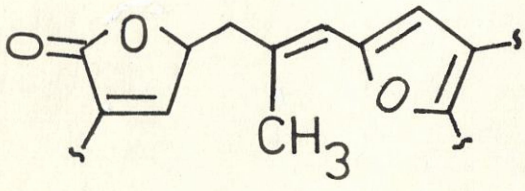
When the signal at δ 5.93 was irradiated, the olefinic methyl at δ 1.56 and a broad singlet at δ 6.31



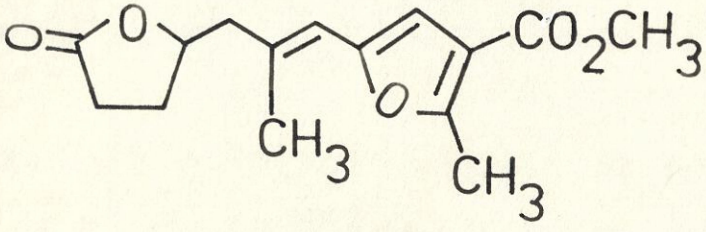








(D)



(XXXVIII)

(1H) sharpened. The resonance at δ 6.31 was attributed to a β-proton in a 1,2,4-trisubstituted furan as in compound F. A second low field signal at δ 7.31 (1H,bs) was assigned to a β-proton on an α-substituted α,β-unsaturated γ-lactone.

The functionalities suggested have accounted for all the oxygen atoms in compound G together with ten of the eleven double bond equivalents required by the molecular formula. This indicated that compound G was monocarbocyclic.

The broad-band ^{1}H decoupled ^{13}C NMR (100MHz) spectrum showed signals for all twenty-one carbons. The SFORD spectrum showed the presence of three methyl carbons (δ 20.2, 22.0, 51.3) and four methylene carbons, of which one was sp^2 hybridized (δ 28.3, 37.6, 40.1, 116.5). In addition, there were five methine carbons (three sp^2 hybridized) at δ 58.6, 78.9, 107.8, 119.8 and 149.5, and nine quaternary sp^2 hybridized carbons (113.8, 127.9, 140.3, 140.3, 148.7, 158.4, 164.2, 173.3, 203.5). These signals could be assigned, in part, to the functional groups already indicated above by other physical measurements as for compound G (see opposite page).

A broad singlet at δ 5.26 (1H) in the ^{1}H NMR (80MHz) spectrum of compound G was assigned to a γ-proton on an α,β-unsaturated γ-lactone. Irradiation of this proton resulted in the sharpening of the signal at δ 7.31 while two doublets of doublets at δ 2.46 (1H,J=14,3Hz) and

TABLE 4

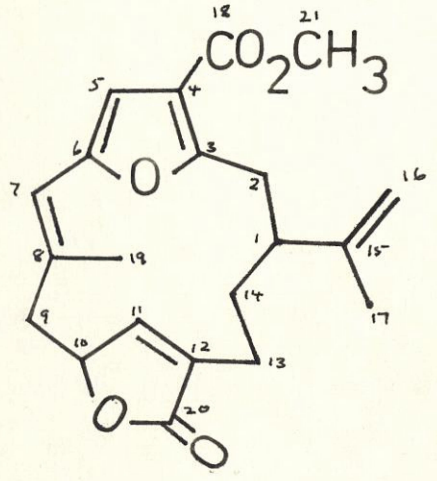
^{13}C NMR data for compounds F*, G*, and Pukalide**

C#	F	G	Pukalide
1	41.5	58.6	40.7
2	31.5	28.3	32.5
3	259.7	158.4	160.0
4	116.0	113.8	113.9
5	110.5	107.9	106.4
6	151.2	148.8	148.2
7	48.5	119.8	55.0
8	80.7	140.3	57.0
9	147.5	40.1[a]	40.0
10	138.0	78.9	77.8
11	22.5	149.5	148.2
12	37.5	127.9	137.3
13	148.0	37.6[a]	32.5
14	111.5	203.5	22.8
15	18.7[a]	140.3	145.8
16	165.0	116.5	112.9
17	142.0	20.2[b]	19.8[a]
18	115.5	164.2	163.8
19	21.0[a]	22.0[b]	18.7[a]
20	176.0	173.3	173.7
21	51.0	51.3	51.2

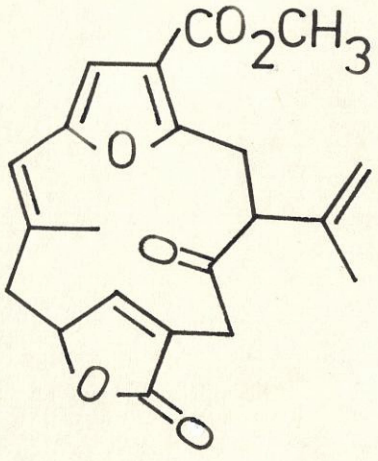
* Recorded at 100MHz in $CDCl_3$ solution.

** Taken from reference 23(a)

a,b Indicates assignments within a column that may be reversed.


18
21
CO_2CH_3
5
4
6
3
O
7
2
16
1
8
19
15
14
17
9
11
10
12
13
O
20
O

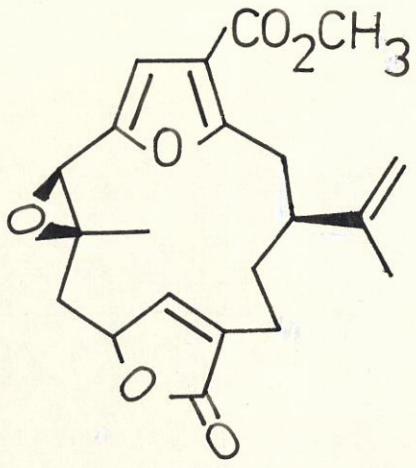
(XXXIX)


CO_2CH_3
O
O
O
O

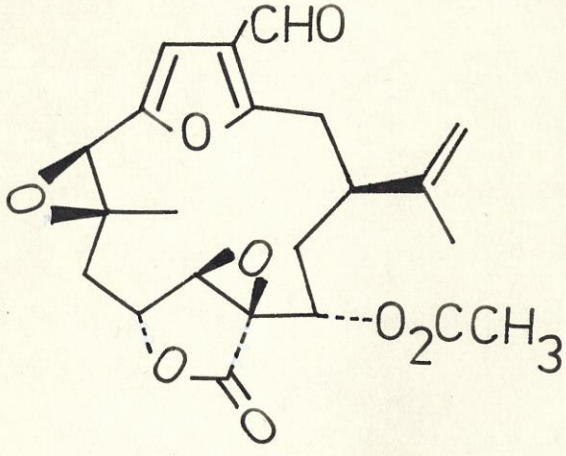
(XL)

2.93 (1H,J=14,4Hz) collapsed into mutually coupled doublets (J=14Hz). This indicated that the γ-lactone in compound G is connected to a methylene group instead of a methine as in compound F. The foregoing data are consistent with partial structure (D). The geometry of the trisubstituted double bond was assumed to be *E* by comparison of the ^{1}H NMR signal [δ 5.93 (1H,bs)] for the olefinic proton with that [δ 6.02 1H,s)] for the model compound (XXXVIII).[23(b)]

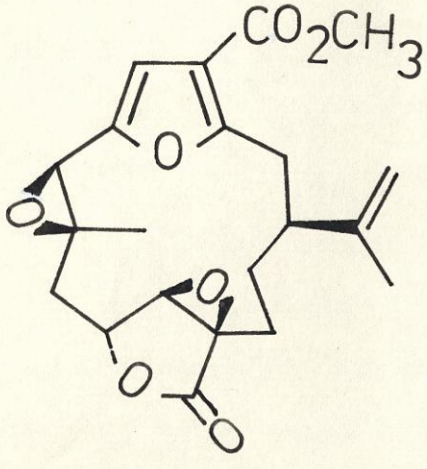
The location of two methylene, one methine and a carbonyl group within a monocarbocyclic framework remained to be elucidated. Biogenetic considerations suggested that compound G was a furanocembranolide of general structure (XXXIX). Since the infrared data indicated that the ketone group in compound G was not conjugated, it could only be located on C-14. An AB spin system with resonances at δ 3.91 (1H,d,J=12Hz) and 4.07 (1H,bd,J=12Hz) in the 400MHz ^{1}H NMR spectrum of compound G were readily accommodated on C-13. The structure (XL) is therefore proposed for compound G. The protons attributed to the C-2 methylene group resonated at δ 3.03 (1H,bd,J=12Hz) and 3.19 (1H,dd,J=16,3Hz) and was coupled to the C-1 proton which had a signal at δ 3.52 (1H,dd,J=16,12Hz). This was supported by the observed downfield shift (δ 58.6) of the ^{13}C resonance for C-1, compared with that (δ 40.7) for pukalide (XLIA) (Chap. 7, Ref. 42).



(XLIA)



(XLIB)



(XLIC)

Compound G represents the fourth member of a small but growing class of furanocembranolides isolated from octocorals. In addition to pukalide (XLIA), (Chap. 7, Ref. 40 - 42) lophotoxin (XLIB) and 11β,12β-epoxypukalide (XLIC) were previously isolated. The co-occurrence of a pseudopterane and a cembrane derivative supports the suggestion by Fenical that pseudopteranes are derived biogenetically from cembranes by a mechanism involving ring contraction.

The natural products are the free acids of the methyl esters, compounds F and G.

5.5 EXPERIMENTAL

Air dried *Pseudopterogorgia acerosa* (200g) was extracted with acetone (2.5 L), and the extract filtered, and concentrated in vacuo. The resulting aqueous suspension (100 mL) was diluted with methanol (400 mL) and extracted with light petroleum (3 x 200 mL). The aqueous methanol layer was diluted with water (200 mL) and extracted with chloroform (3 x 200 mL). Removal of the solvent from the chloroform extract, yielded a dark red gum (7.3g, 3.65%).

The chloroform extract (4.5g) was subjected to short column chromatography using light petroleum-ethyl acetate mixtures as eluting solvents. Elution with 75% ethyl acetate gave a fraction (520 mg) which was treated with excess ethereal diazomethane, and subjected to PLC [light petroleum-ethyl acetate (3:1)] to give, in order of decreasing R_f values, bands A, B, and C. Band A was eluted and recrystallized from light petroleum-acetone to give compound F (XXXVI) (60 mg, 0.03% based on the dried gorgonian).

COMPOUND F (XXXVI)

Compound F (XXXVI) , m.p. 130 - 132°

$[\alpha]_D^{22}$ + 62° : (c 0.225, $CHCl_3$)

λ_{max} : 245 nm (ε 6000)

ν_{max} ($CHCl_3$) : 3080, 1760, 1720, 1640, 1610, 1579, and 895 cm^{-1}

^1H NMR (360MHz) : δ 0.84 (1H,ddd,J=14,14,3Hz), 1.80 (1H, bs), 1.81 (3H,bs), 1.99 (3H,bs), 2.21 (1H,bd,J=14Hz), 2.44 (1H,ddd,J=14,14, 3Hz), 2.73 (1H,d,J=16Hz), 2.78 (1H,m), 3.32 (1H,dd,J=16,12Hz), 3.83 (3H,s), 3.84 (1H,m), 4.82 (1H,bs), 4.86 (1H,bs), 5.06 (1H,bs), 5.07 (1H,bs), 5.44 (1H,dd, J=4,1Hz), 6.39 (1H,bs), 6.75 (1H,bs) ppm.

^{13}C NMR (100MHz): δ 18.7(q), 21.0(q), 22.5(t), 31.5(t), 37.5(t), 41.5(d), 48.5(d), 51.0(q), 80.7(d), 110.5(d), 111.5(t), 115.5(t), 116.0(s), 138.0(s), 142.0(s), 147.5(d), 148.0(s), 151.2(s), 159.7(s), 165.0(s), 176.0(s) ppm.

Mass spectrum : m/z 206(56), 188(19), 177(5), 163(14), 161(14), 151(64), 135(15), 122(5), 115(11), 105(5), 91(100). Chemical ionisation mass spectrum m/z 374 $[M + NH_4]^+$

Band B (30 mg) consisted of a mixture of at least three compounds, and was not investigated further.

Band C was eluted and the solvent evaporated to give compound G (XL) as a yellow, moderately unstable oil (20 mg, 0.01%).

COMPOUND G (XL)

$[\alpha]_D^{22}$ + 35°	:	(c 0.425, $CHCl_3$)
λ_{max}	:	253 nm (ε 6800)
ν_{max} ($CHCl_3$)	:	3080, 3020, 1760, 1715, 1710, 1640, 1600, 1550, and 905 cm^{-1}
^1H NMR (400MHz)	:	δ 1.56 (3H,d,J=1.5Hz), 1.79 (3H,t, J=1Hz), 2.46 (1H,dd,J=14,3Hz), 2.93 (1H,dd,J=14,4Hz), 3.03 (1H,bd,J=12Hz), 3.19 (1H,dd,J=16,3Hz), 3.52 (1H,dd, J=16,12Hz), 3.81 (3H,s), 3.91 (1H,d, J=12Hz), 4.07 (1H,nd,J=12Hz), 5.05 (1H, bs), 5.08 (1H,bs), 5.26 (1H,bs), 5.93 (1H,bs), 6.31 (1H,bs), 7.31 (1H,bs) ppm.
^{13}C NMR (100MHz):		δ 20.2(q), 22.0(q), 28.3(t), 37.6(t), 40.1(t), 51.3(q), 58.6(d), 78.9(d), 107.8(d), 113.8(s), 116.5(t), 119.8(d), 127.9(s), 140.3(s), 140.3(s), 148.7(s), 149.5(d), 158.4(s), 164.2(s), 173.3(s), 203.5(s) ppm.

Mass spectrum : m/z 370 (M+ 21%), 339(7), 311(2), 246(37), 245(16), 192(68), 191(70), 187(16), 186(15), 133(65), 104(24), 43(100)

HRMS (Found 370.1447, calcd. for $C_{21}H_{22}O_6$: 370.1416).

CHAPTER SIX

AN INVESTIGATION INTO THE CONSTITUENTS OF *PLEXAURA* SP.

6.1 INTRODUCTION

Chemical investigation of the gorgonian *Plexaura homomalla* by Weinheimer and Spraggins[25] resulted in the isolation of relatively high concentrations (1.5% of the dried weight) of the prostaglandins 15-*epi*-PGA_2 (XLII) and its diester (XLIIB). Some forms of *P. homomalla* were subsequently shown to contain derivatives of 15*S*-PGA_2 and 15*S*-PGE_2 that are identical with mammalian prostaglandins.[26] More recently, Ravi and Wells[27] isolated six unusual lipids (XLIII - XLVIII) and two compounds (XLIX and L) of mixed biogenesis from *P. flava*. In contrast, Ciereszko and his co-workers[28] isolated the cembrane diterpenoid plexaurolone (LIA) from a *Plexaura* sp. that closely resembles *P. homomalla*.

Investigation of an unidentified *Plexaura* sp. obtained locally, resulted in the isolation of plexaurolone and related diterpenoids.

6.2 EXTRACTION

Dried, minced *Plexaura* sp. was extracted with dichloromethane, and the solvent evaporated to give a viscous brown oil in 4.6% yield.

The oil was chromatographed on a short column, and fractions eluted with light petroleum-chloroform (3:1), and continued with increasing concentrations of chloroform, followed by ethyl acetate. Combination of similar fractions after monitoring by TLC, gave three major fractions (A, B, and C).

Fraction A contained mainly lipids and was not investigated further. Fraction B was subjected to PLC [light petroleum-acetone (3:1)], and three bands were recovered after viewing the chromatoplates under long wavelength UV light. The residues obtained on elution of each band were separately crystallized from light petroleum-acetone to give, in order of increasing R_f values, compounds H, I, and J. Fraction C mainly consisted of an unstable diol and was not investigated further.

Examination of a crude mixture of plexaurolones (from *Plexaura* sp.[28]) kindly provided by Professor L. Ciereszko was shown to contain plexaurolone (LIA), an unstable diol, and a ketodiol (compound K). The structure of compound K was determined by single crystal X-ray diffraction analysis.

6.3 STRUCTURE AND STEREOCHEMISTRY OF COMPOUNDS H, I, AND J

COMPOUND H

Compound H was isolated as white needles, m.p. 110 - 111°, $[\alpha]_D$ - 8.3° (c 0.21, $CHCl_3$). The molecular formula $C_{20}H_{34}O_3$ (M+ 322) was established for compound H by a combination of mass spectrometry and ^{13}C NMR spectroscopy. Compound H did not contain any significant UV absorption. The IR spectrum had absorption bands for hydroxyl (3440 cm^{-1}), ketone (1700 cm^{-1}), and terminal olefinic (3080, 1640, and 880 cm^{-1}) groups.

Compound H had resonances in its ^1H NMR spectrum at δ 0.94 (3H,d,J=6Hz), 1.00 (3H,d,J=6Hz), and 1.04 (3H,d, J=6Hz), due to three methyl groups, and at δ 1.68 (3H,bs) assignable to an olefinic methyl group. Irradiation of the signal at δ 1.68 caused a broad singlet at δ 4.71 (2H) to sharpen, thereby establishing the presence of an isopropenyl group in compound H. A resonance at δ 3.36 (1H,m) was attributed to the carbinyl proton of a secondary alcohol. This was confirmed by the formation of an acetate, m.p. 92 - 94°, in which the carbinyl proton appeared at δ 4.70 (1H, m). Compound H acetate had the formula $C_{22}H_{36}O_3$ (M+ 364, 3%) as established by high resolution mass spectrometry (obsd. 304.2396, M-CH_3CO_2H; calcd. 304.2402).

The ^{13}C NMR spectrum of compound H (Table 5) showed resonances for twenty carbons. Four methyl groups had resonances at δ 13.2 (q), 15.9(q), 20.5(q), and 21.0(q). A signal at δ 70.7(d) was readily assigned to the carbon of a secondary alcohol while resonances at δ 211.3(s), and 214.4(s) were attributed to two ketone groups. The olefinic carbons in compound H had resonances at δ 109.9(t), and 148.8(s). The above spectral assignments accounted for three of the four degrees of unsaturation indicated for compound H by the molecular formula. Compound H is therefore, monocarbocyclic.

The spectral features of compound H suggested that is was a cembrane derivative with a saturated 14-membered carbocycle. Examination of the literature revealed that compound H was identical with plexaurolone (LIA)[28] (m.p. $[\alpha]_D$, IR, ^1H and ^{13}C NMR, and MS).

COMPOUND I

Compound I, m.p. 93 - 95°, $[\alpha]_D$ + 48° (c 0.27, $CHCl_3$) had the molecular formula $C_{20}H_{32}O_3$ (HRMS: M+ obsd. 320.2361, calcd. for $C_{20}H_{32}O_3$: 320.2351). The IR spectrum showed absorptions for ketone (1705 cm^{-1}) and olefinic (3080, 1645, 890 cm^{-1}) groups.

TABLE 5

^{13}C NMR data for compounds H - J

C#	H*	I+	J+
1	47.9	47.2	47.3
2	39.2	46.4	46.8
3	70.7	215.6	215.3
4	37.1	41.4[a]	41.2[a]
5	48.3[a]	49.5[b]	50.9
6	211.3	210.4	210.8
7	48.2[a]	47.4[b]	47.4
8	27.4	29.5	27.1
9	31.3	32.1	32.1
10	36.4	36.6	38.3
11	214.4	214.4	214.6
12	43.2	41.5[a]	42.6[a]
13	29.4[b]	29.9[c]	31.3[b]
14	29.5[b]	31.6[c]	30.7[b]
15	148.8	147.6	147.5
16	20.3	20.1	20.4
17	109.9	110.6	110.6
18	15.9	16.4[d]	16.5[c]
19	20.5	21.4	22.1
20	13.2	17.3[d]	17.3[c]

* Recorded at 20MHz in $CDCl_3$ solutions. Assignments are based upon J-Modulated Spin Echo experiments.

\+ Recorded at 100MHz in $CDCl_3$ solutions. Multiplicities were obtained by single-frequency off-resonance decoupling.

a - d Signals within a column may be reversed.

The ^{1}H NMR spectrum of compound I was similar to that of compound H (LIA). Thus, resonances at δ 1.00 (3H,d,J=6Hz), 1.03 (3H,d,J=6Hz), 1.04 (3H,d,J=6Hz), and 1.68 (3H,bs), were attributed to three saturated and one olefinic methyl groups, respectively. The resonance at δ 1.68 was coupled (allylic) to a broad singlet at δ 4.72 (2H), revealing the presence of an isopropenyl group in compound I.

The ^{13}C NMR spectrum of compound I showed resonance at δ 210.4(s), 214.4(s), and 215.6(s), due to the presence of three ketone groups. Signals at δ 110.6(t) and 147.6(s) were readily assigned to a terminal olefin. The spectral data presented above indicated that compound I was an oxidation product of plexaurolone (LIA). This was confirmed by the oxidation of (LIA) with pyridinium chlorochromate (PCC) to yield a product that was identical with compound I (including optical rotation) in 64% yield.

Compound I (LII), designated as oxyplexaurolone is the second member of a new group of cembrane derivatives in which the 14-membered carbocycle is saturated.

COMPOUND J

Compound J crystallized as white needles, m.p. 196 - 199°, $[\alpha]_D$ - 11° (c 0.09, $CHCl_3$). The molecular formula

$C_{20}H_{32}O_3$ (HRMS: M+ obsd. 320.2364, calcd. 320.2351) indicated that compound J was isomeric with compound I. The IR spectrum of compound J showed absorptions for ketone (1705 cm^{-1}) and olefinic (1645, 890 cm^{-1}) groups.

The ^1H NMR spectrum of compound J had resonances at δ 0.93, 0.99 and 1.04 (all 3H,d,J=6Hz), and 1.68 (3H, bs) due to three methyls and one olefinic group, respectively. Signals at δ 4.72 (1H,bs) and 4.73 (1H,bs) were assigned to a terminal olefin.

The ^{13}C NMR spectrum of compound J was similar to that of compound I (Table 5). Signals for three ketone groups resonated at δ 210.8(s), 214.5(s), and 215.3(s). Resonances at δ 110.6(t) and 147.5(s) were assigned to a terminal double bond.

The mass spectral fragmentation pattern (see experimental) of compounds I and J were virtually identical, suggesting that both compounds differed from each other only in stereochemistry. Attempts to epimerise compound I were unsuccessful. However, epimerisation of compound J with 2% potassium hydroxide in methanol resulted in a mixture of compounds I and J (TLC, ^1H NMR). This indicated that compounds I and J differ in stereochemistry at C-4 or C-12. Careful comparison of the ^1H NMR data (Table 6) of plexaurolone and its derivatives indicated that the resonances

TABLE 6

Selected ^{1}H NMR data for compounds H - K

C-Me	H*	H-acetate**	I**	J**	K*
4	0.97+	0.95	1.03	0.93	0.97
8	1.04	1.04	1.04	1.04	1.07
12	1.00	1.00	1.00	0.99	0.97

* Recorded at 80MHz

** Recorded at 200mHz

\+ All signals were doublets, J=6Hz

for C-8 Me and C-12 Me usually appear at ~ δ 1.00 and 1.04, respectively. The resonance for C-4 Me was the only variable methyl doublet in the 14-membered carbocycle. For compounds I and J the C-4 Me resonated at δ 1.03 and 0.93, respectively. On the basis of the foregoing evidence, structure (LIII) is proposed for compound J.

6.4 STRUCTURE AND STEREOCHEMISTRY OF COMPOUND K

Examination of a crude diterpenoid mixture from *Plexaura* sp.,[28] kindly provided by Professor L. Ciereszko, revealed the presence of plexaurolone (LIA), an unstable diol (identical to the one present in our sample), and a ketodiol, designated as compound K.

Compound K was isolated as colourless prisms, m.p. 125 - 126°, $[\alpha]_D$ + 42° (c 0.12, $CHCl_3$). The molecular formula of compound K, $C_{20}H_{36}O_3$ (324.50) was established by x-ray diffraction analysis. The IR spectrum showed absorptions due to hydroxyl (3420 cm^{-1}), ketone (1705 cm^{-1}), and olefinic (1645, 880 cm^{-1}) group.

The ^1H NMR spectrum of compound K showed resonances at δ 0.97 (6H,d,J=6Hz), 1.07 (3H,d,J=6Hz), and δ 1.75 (3H,t,J=1Hz), assignable to three methyls, and one olefinic methyl groups, respectively. Irradiation of the resonance at δ 1.75 caused signals at δ 4.67 (1H,bs), and

4.70 (1H,bs) to sharpen, thereby establishing the presence of an isopropenyl group in compound K. Attempts to form an acetate of compound K or to oxidize the hydroxyl groups resulted in a facile intramolecular reaction to form an ether bridge (unpublished results).

The structure of compound K was determined by a single-crystal X-ray analysis, kindly performed by Mr. Louis Todaro of Hoffmann-La Roche Inc., New Jersey. What follows was included for completeness. "The unit cell contains two independent molecules (designated as unprimed and primed) that is, two molecules not related by independent symmetry. Perspective drawings of these two independent molecules are shown in Figures I and II. For approximately 75% of the primed molecules in the crystal, the carbon atoms at C-9 and C-10, have the orientation shown in Figure I; while for the remaining 25%, they are oriented as in Figure II. The two independent molecules are identical in configuration but differ in conformation. Conformation differences become apparent by examining the 14-membered ring torsion angles listed in Table 5 of the supplementary X-ray crystallographic data.

The crystal data for compound K are summarised in Table 7. The intensity data were measured on a Hilger-Watts diffractometer (Ni-filtered Cu Kα radiation, θ - 2θ scans, pulse-height discrimination). The size of the crystal used for data collection was approximately 0.20 x 0.5 x 0.9 mm;