FINAL REPORT

ISOLATION AND STRUCTURE ELUCIDATION OF NATURAL PRODUCTS FROM MARINE ORGANISMS AND DETERMINATION OF THEIR BIOLOGICAL ACTIVITY

Research Grant No. RG - 85 - C - 06

FINAL REPORT

Grant No	:	RG/85/C/06
Title of the Research Project	:	Isolation and structure Elucidation of Natural Products from Marine Organisms and Determination of their Biological Activity
Date of Award of the Grant	:	15 March 1985
Grantees	:	Professor L.M.V. Tillekeratne Dr (Mrs) S.A. Deraniyagala Department of Chemistry University of Colombo
Research Assistant	:	J.A. Chandrasiri (15 March 1985-02 October 1988)
Out put	:	Publications (Annexture 4) J.A. Chandrasiri has registered for the degree of M.Phil (Organic Chemistry) at the University of Colombo on the basis of his work on the above project.

CONTENTS

1. GENERAL INTRODUCTION

1.1 Secondary Metabolites in Marine Organisms

Marine organisms are a rich source of novel and interesting secondary metabolites such as terpenoids, steroids, alkaloids, prostaglandins, fatty acid derived lipids and peptides etc. The commonest among these are the terpenoids, which can be classified as monoterpenes, sesquiterpenes, diterpenes, sesterterpenes and triterpenes. The classes of terpenoids present vary from organism to organism. For example marine organisms like soft corals (Phylum Coelenterata) contain mainly diterpenoids while sponges (Phylum Porifera) contain mainly sesquiterpenoids.

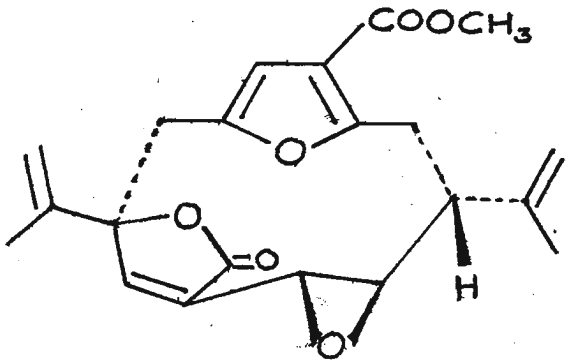
Furanoterpenes are an interesting group of metabolites isolated from marine organisms. Furanoterpenes in soft corals, compared to marine sponges, have a relatively restricted distribution. Only a few furanoterpenoids have so far been isolated from the soft corals.

1.1.1 Furano-cembranoid Diterpenes

Only a relatively small number of furano-cembranoid diterpenes have been isolated from marine organisms todate.

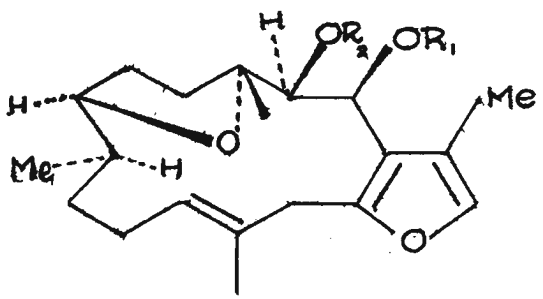
One of these compounds is Pseudopterolide (1) isolated from the Caribbean sea whip Pseudopterogorgia acerosa[1] (Gorgonacea). Although this interesting compound does not have a 14-memberd

cembranolide skeleton, it showed cytotoxic activity, by inhibiting overall cell cleavage of fertilized urchin eggs.



(1)

Bowden et al have isolated three furano-cembranoid diterpenes, in which the isopropyl groups are incorporated in the furan ring. Pachyclavulariadiol (2) and its mono and diacetylated derivatives (3,4) have been isolated from the soft coral Pachyclavularia violacea[2] (= erecta) (Clavulariidae, Stolonifera).

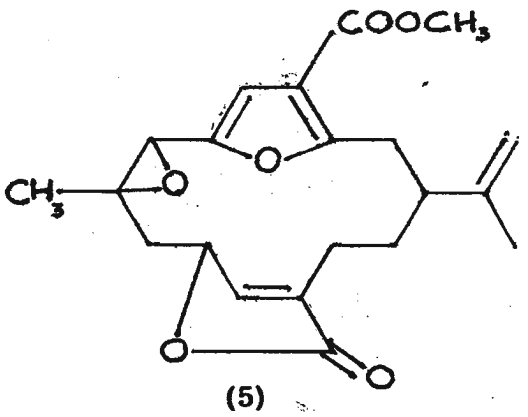


(2) $R_1 = R_2 = H$

(3) $R_1 = Ac, R_2 = H$

(4) $R_1 = H, R_2 = Ac$

The isolation of pukalide (5), the first furano-cembranoid diterpene, having α,α'-disubstituted furan-β-carboxylate and epoxy functions was first reported by Scheuer et al in 1975 from the Hawaiian soft coral Sinularia abrupta[3].



(5)

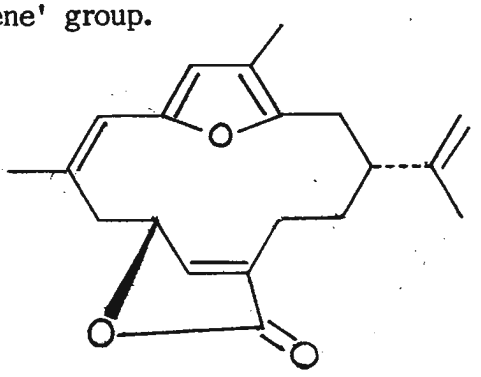
Later, in 1985 and 1987, pukalide has been isolated from the CH_2Cl_2 extract of the soft coral Sinularia imbriolobata[4] collected from Jaffna in the nothern coast of Sri Lanka and from the skin extract of the dendranotoid nudibranch Tochuina tetraquetra[5] collected from Port Hardy in British Columbia, respectively.

However, no exact explanation had been offered so far for the absence of pukalide from the same nudibranch sp. collected from a different locality, Bamfield, British Columbia.[5]

Soft corals and nudibranchs are not the only sources of pukalide. This interesting furano-diterpenoid has been isolated from gorgonian species as well. M.M. Bandurraga in 1982 reported the presence of pukalide in the tropical gorgonian Lophogorgia rigida.[6]

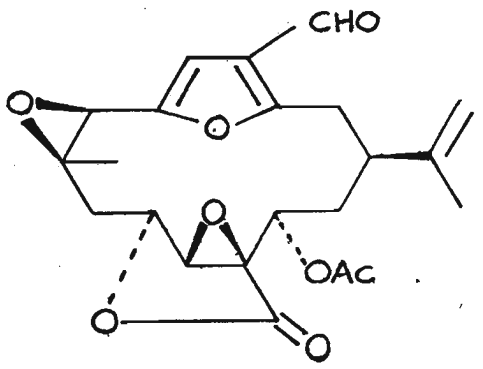
From the soft coral Gersemia rubiformis, Williams et al isolated another furano-cembranoid diterpene, rubifolide (6)[7] with an unusual α,α'- dialkyl -β- furan methyl group instead of α,α' - dialkyl - β -

furan carboxylate function. In addition, the epoxy group has been replaced by an 'ene' group.



(6)

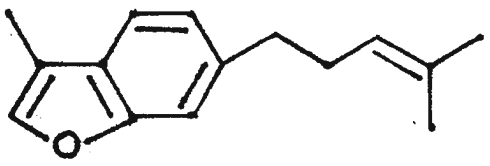
Another furano-cembranoid, lophotoxin (7)[8], a paralytic agent that binds to a nicotinic receptor subunit has been isolated from the from the Lorphogorgia rigida. The LD_{50} of lophotoxin was shown to be 8mg/kg.[9] It is being used as an important neuropharmacological probe in neurobiology.[10]



(7)

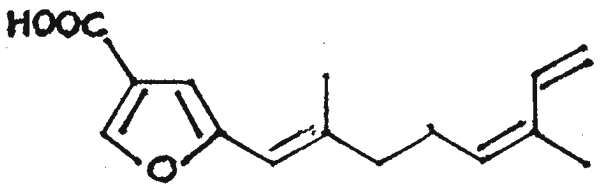
1.1.2. Furano-sesquiterpenes

Compared to maring sponges, a relatively small number of furano-sesquiterpenes have been isolated from the soft corals. One of these compounds is furoventalene (8) isolated from a gorgonian, Gorgornia ventalina by Weinheimer.[11] It showed anti-virul activity.



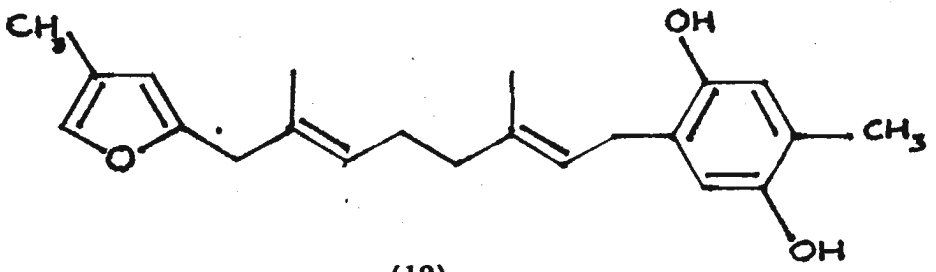
(8)

In 1977, Coll and co-workers isolated a furano-sesquiterpene acid (**9**) from the soft coral Sinularia gonatodes.[12] Although a majority of sesquiterpenes contained β-substituted furan rings, this compound had a α- substituted furan ring.



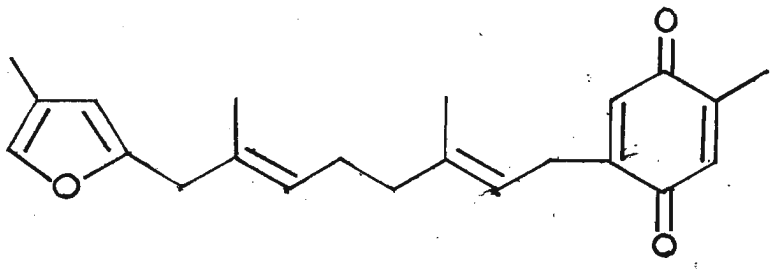
(9)

It is important to note that most of the furano-sesquiterpenes isolated from marine sponges are oils. Most of the furano-sesquiterpenes isolated from soft corals are crystalline compounds. The sesquiterpene acid (**9**) has some structural similarity to prenylated quinol (**10**) obtained from the soft coral Sinularia species.[13]



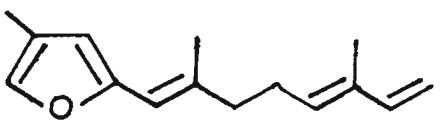
(10)

In this compound also, the polyprenyl chain is present at the α- carbon of the furan ring. The related compound (**11**) having a quinone moiety has also been isolated by Coll and co-workers.[14]



(11)

In addition to (**10&11**), several other furanosesquiterpenes (**12-23**) have been isolated from Sinularia capillosa,[15] all of which are structurally related to the furanosesquiterpene acid (**9**).



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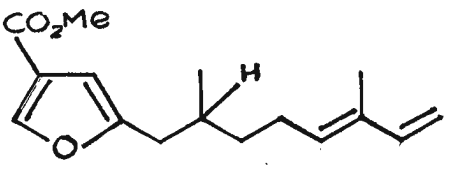

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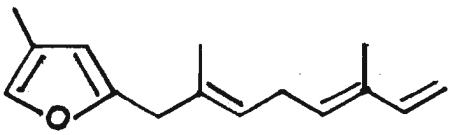

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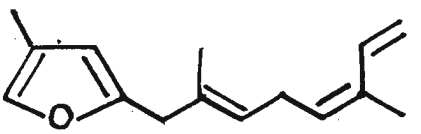

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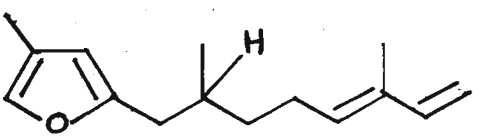

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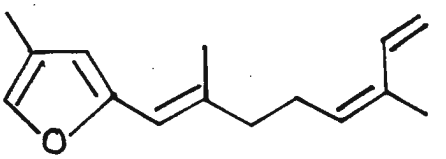

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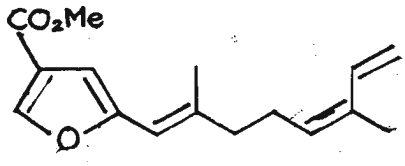

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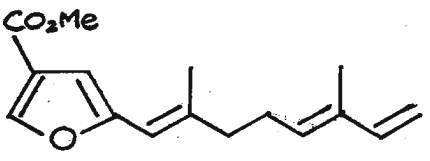

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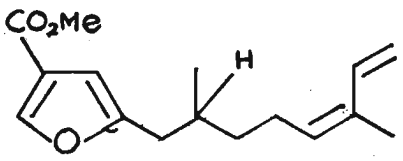

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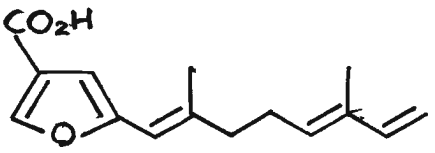

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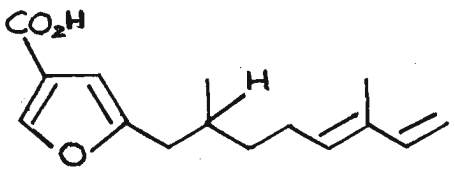

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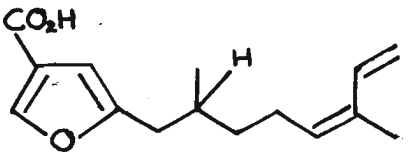
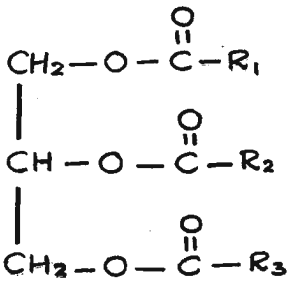


(23)

1.1.3 Fatty Acid Derived Lipids

Fatty acids are very common biological constituents and they are generally considered to be non toxic. However, sometimes they show toxicity because of their strong surface active properties. Marine organisms have a large number of fatty-acid derived compounds. For example, triglycerides (**24**) having the most common fatty acids such as palmitic, stearic and oleic acids, wax esters (**25**), diacyl glyceryl ethers (**26**), and glycerol ethers (**27**) from marine fish and soft corals,[16] fatty acids possessing chain length C-24 to C-28 (eg. 28 & 29) from marine sponges[17,18] and several anti-bacterial metabolites like chlorellin and polyunsaturated fatty acids from marine algae[19] have been isolated.



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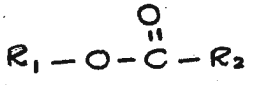

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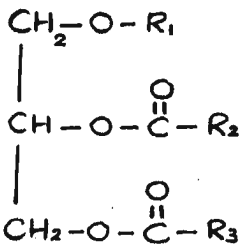

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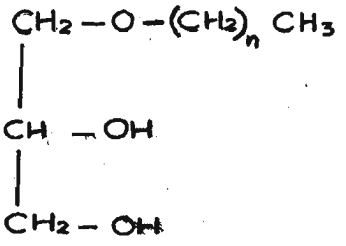

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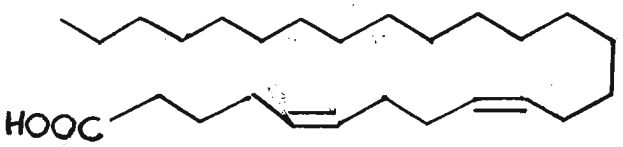

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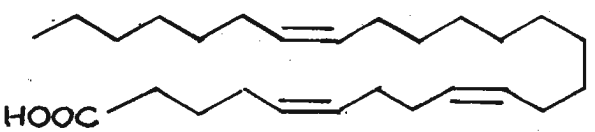
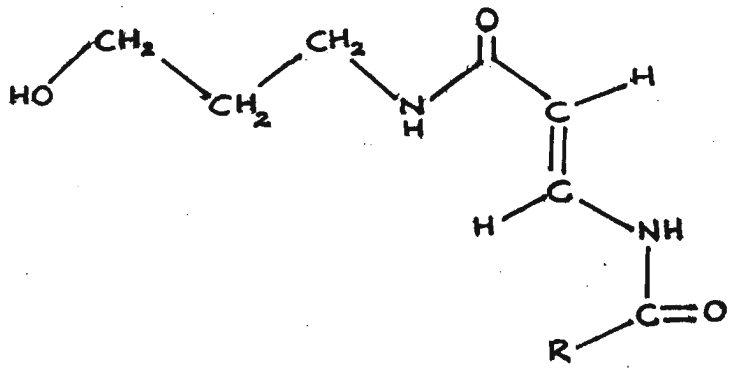


(29)

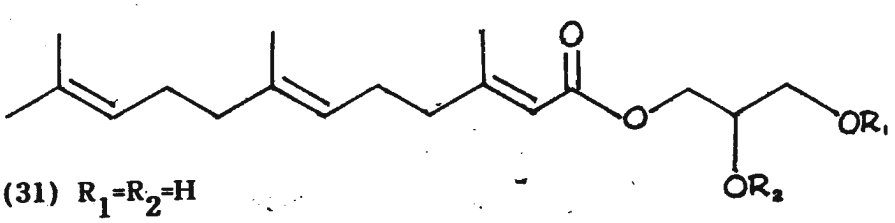
Palytoxin (**30**), a marine toxin, which has the molecular formula $C_{145}H_{264}N_4O_{78}$ was first isolated in 1971 by Moore and Scheuer from Palythoa spp. of the family Zoanthidae. This compound has a very complex structure and so far only a fragment of the structure has been assigned as N-hydroxypropyl - β-amidoacrylamide.[17]



(30)

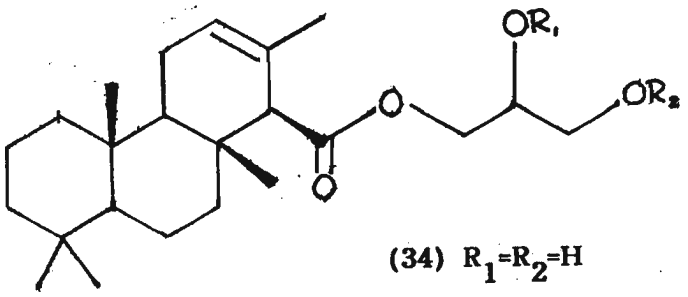
The lipid functionality of this toxin is still unknown. Moore and Scheuer reported the LD_{50} of palytoxin in mice as 0.15µg/kg while Wiles et al reported that palytoxin which was isolated from another species Palythoa vestitus, has the LD_{50} activity ranging from 0.033-0.45 µg/kg on test animals.[17] This powerful toxin, which has anticarcinogenic activity was found to be active in cardiovascular systems , and induces the contraction at a minimum concentration of 10^{-10} g/ml in the blood vessels of various animals.[17]

In 1980, three terpenoic acid glycerides, farnesic acid glyceride (**31**) and its two monoacetylated derivatives (**32**) and (**33**) have been reported from the nudibranch Archidoris odhneri.[20]



(31) $R_1=R_2=H$
(32) $R_1=Ac$, $R_2=H$
(33) $R_1=H$, $R_2=Ac$

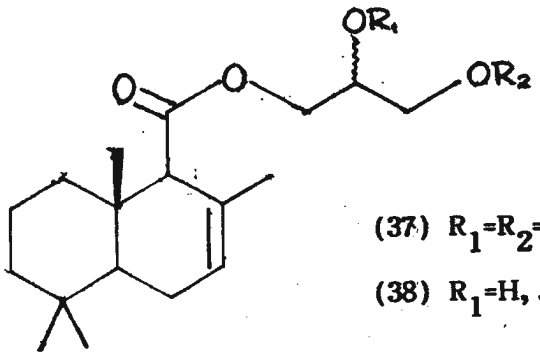
Among the marine nudibranchs, Archidoris montereyensis has yielded a series of glyceride derivatives.[21] The diterpenoic acid glyceride (**34**), its monoacetates (**35**) and (**36**), drimane sesquiterpenoic acid glyceride (**37**), the monoacetate (**38**), the monocyclofarnesic acid glyceride (**39**) and 1-O-hexadecylglycerol (**40**) have been isolated from this species.



(**34**) R_1=R_2=H

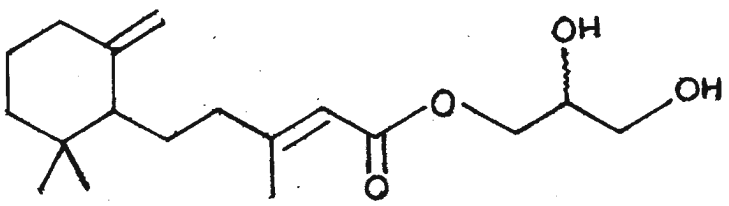
(**35**) R_1=H, R_2=Ac

(**36**) R_1=Ac, R_2=H

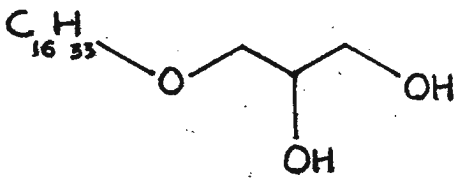


(**37**) R_1=R_2=H

(**38**) R_1=H, R_2=Ac



(**39**)



(40)

The compounds (**34**), (**37**) and (**39**) too have been isolated from Archidoris obhneri as minor metabolites.[21] The drimane sesquiterpenoic acid glyceride (**37**) and the glyceryl ether (**40**) showed fish antifeedent activity against Oligocottus maculosus.[21] The glyceryl ether (**40**), which showed strong anti-bacterial activity against Staphylococcus aureus and Bacillus subtilis has been found as a minor component in the dorid nudibranch Aldisa sanguinea cooperi and in the sponge Halichondria panicea, on which Archidoris montereyensis feeds.[21]

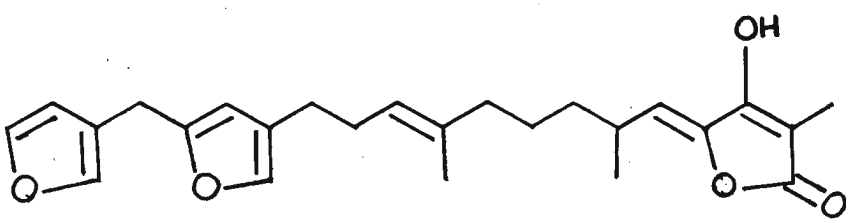
1.1.4. Furano-sesterterpenes from the Marine Sponges

Marine sponges are a fascinating target for chemical investigation of biologically active furanosesterterpenes. Furano-sesterterpenes exhibitting a range of biological activity have been isolated from marine sponges.

Compared to other types of furano-terpenoids, a relatively small number of furano-sesterterpenes have been isolated from marine sponges so far. They can be divided into three main groups ;

linear furano-sesterterpenes with conjugated tetronic acid moiety, linear furano-sesterterpenes with unconjugated tetronic acid moiety and linear furano-sesterterpenes without tetronic acid moiety. Among the compounds in the first and the second groups are a few difuranosesterterpenes. Since the majority of sesterterpenes which showed biological activity have this tetronic acid functionality, it was suggested that the activity was due to the presence of a tetronic acid functionality in the molecule.

Two of the very first compounds reported which belonged to the first group of furano-sesterterpenes were the isomeric ircinin-1 (**41**) and ircinin-2 (**42**) isolated from both Ircinia oros[22] and Cacospongia scalaris.[23]



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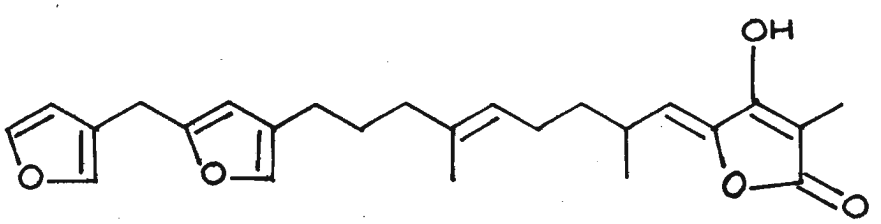
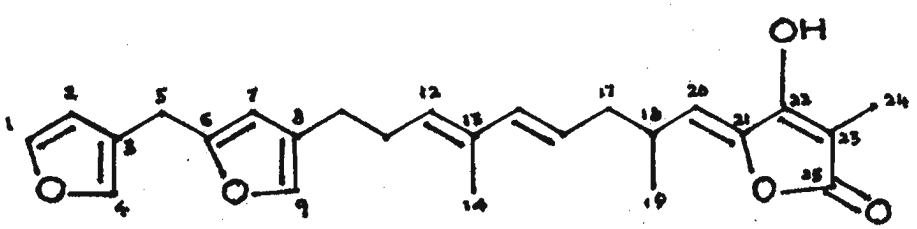


(42)

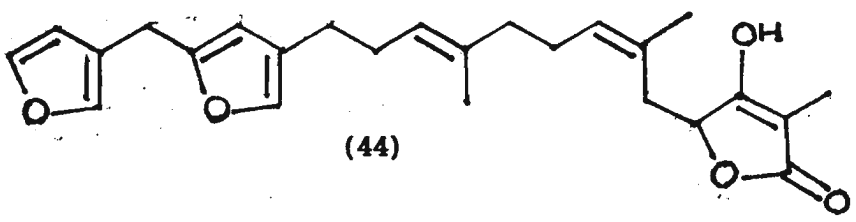
An 1 : 1 mixture of (**41**) & (**42**) reduced the motility of sea urchin sperm at 16µg/ml level.[24]

A double bond derivative (**43**) of the ircinins which showed anti-cell division activity on the fertilized star fish egg at 1.0 µg/ml has been isolated from the marine sponge Cacospongia scalaris.[23] It also showed anti-bacterial activity against Staphylococcus aureus, P. aeruginosa, B. subtilis and M. smegmatis.[23] Although several furanosesterterpenes with conjugated tetronic acid moiety have been isolated from marine sponges of the family Thorectidae, this was the first reported isolation of this type of compound from the genus Cacospongia.



(43)

Two other difurano-sestertepenes lacking the double bond between C-20 and C-21 in the tetronic acid moiety have been isolated from a sponge, Spongionella species by Kato et al[25] in 1985. These two compounds are known as spongionellin (**44**) and dehydrospongionellin (**45**).



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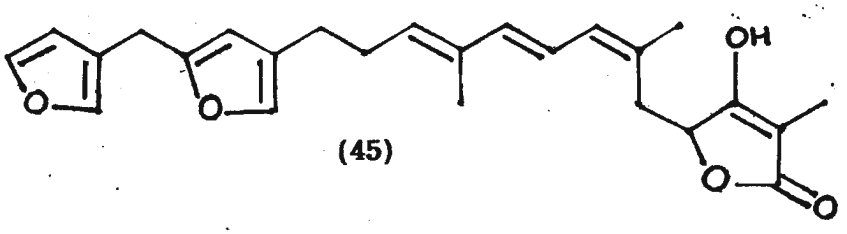
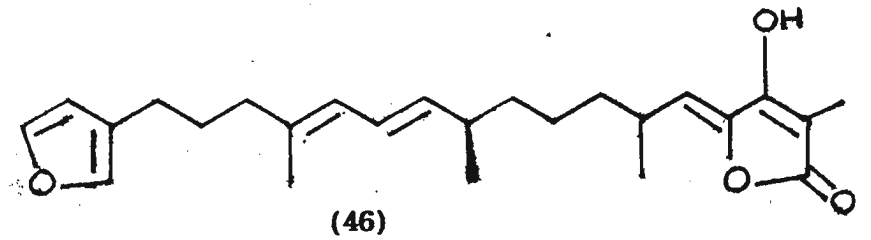
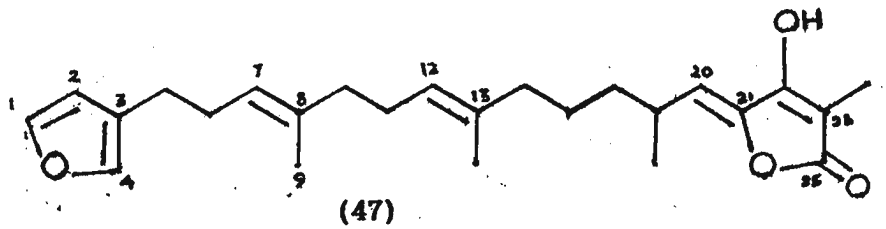


(45)

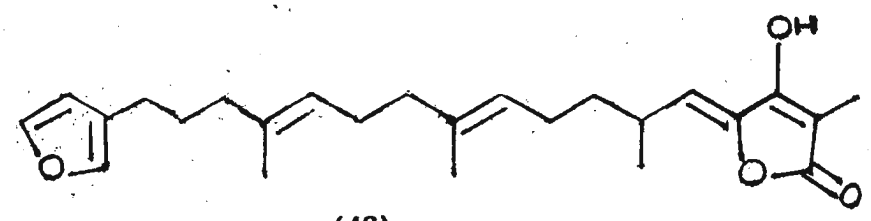
Most of the furanosesterterpenes with conjugated tetronic acid moiety have been isolated from sponges of the genus Ircinia. Among them, fasciculation (**46**)[26], variabilin (**47**)[27] and strobilinin (**48**)[28] are some of the interesting compounds isolated from I. fasciculata, I. variabilis and I. strobilina respectively.



(46)



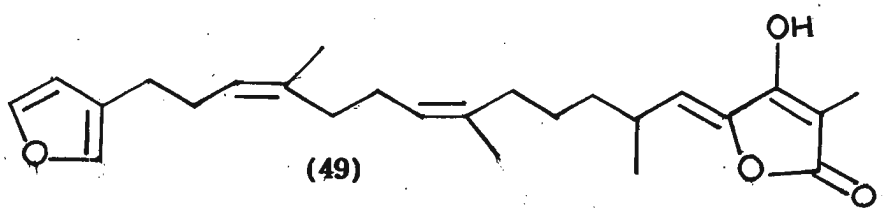
(47)



(48)

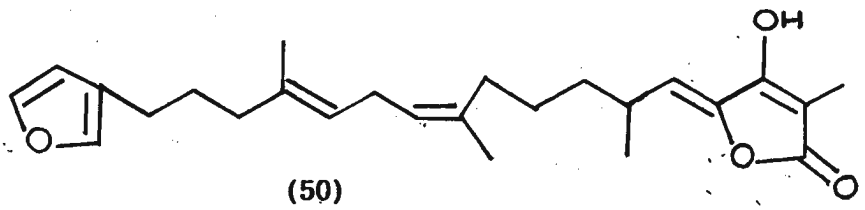
Of these furano-sesterterpenes, variabilin which showed strong anti-bacterial activity against Staphylococcus aureus[27] and anti-cell division activity on sea urchin eggs[29], is one of the most interesting compounds isolated from the genus Ircinia. This compound was first isolated by Faulkner in 1973 and, after 10 years, the stereochemistry at $\Delta^{7,8}$ and $\Delta^{12,13}$ was established by Gonzalez Gonzalez et al.[30], using a sample isolated from I. dendroides.

An E steroechemistry at $\Delta^{7,8}$ and Z stereochemistry at $\Delta^{12,13}$ in variabilin was assigned on the basis of the spectral data (^{1}H-nmr, and ^{13}C-nmr).[30] The stereochemistry at $\Delta^{20,21}$ has not yet been determined. Therefore a more descriptive structure of variabilin is given below. (**49**)



(**49**)

The same research workers isolated the compound (**50**)[30] which is a double bond isomer of variabilin from the same Ircinia species.

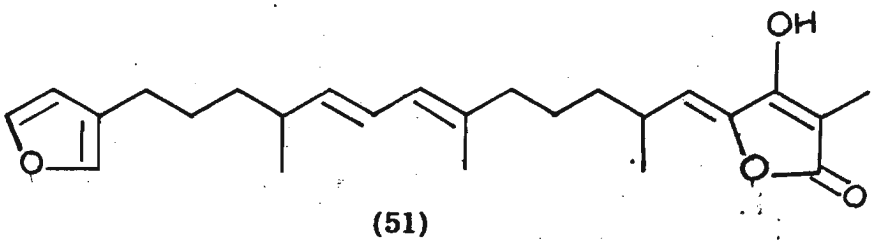


(**50**)

Strobilinin (**48**), the other double bond isomer of variabilin which co-existed with the latter in the ration of 1 : 3 was obtained from the ether extract of I. strobilina, and showed anti-bacterial activity against Staphylococcus aureus and Bacillus subtilis at a minimum inhibition concentration of 3-6 ppm.[28]

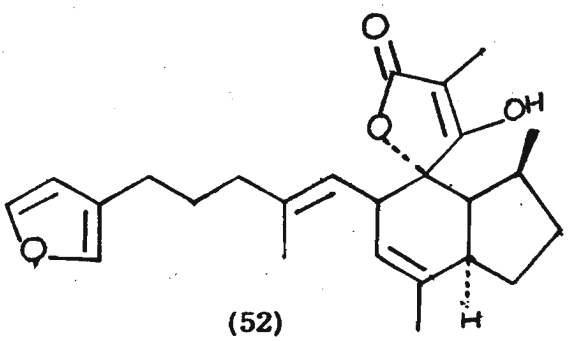
In addition to those isolated from members of the Ircinia genus, the compound (**51**), a furanosesterterpene with tetronic acid functionality has been identified from the sponge Cacospongia scalaris, belonging to the genus Cacospongia.

This compound is a double bond isomer of fasciculatin (**46**) and showed anti-cell division activity on fertilized starfish (Asterina pectinifera) eggs at 1.0 μg/ml as well as anti-bacterial activity against Staphylococcus aureus, B. subtilis, and M. smegmatis.[23]



(51)

In addition to the linear furanosesterterpenes, rare cyclic tetronic acid sesterterpenes have been isolated from the genus Ircinia. Ircinianin (**52**) is such a compound having tricyclic spirotetronic acid functionality.[31]



(52)

Since these furanosesterterpenes are involved in the defence mechanism of the sponges, in addition to isolation and structure elucidation, many attempts have been made to synthesize these active compounds with a view to understanding the defence mechanisms.

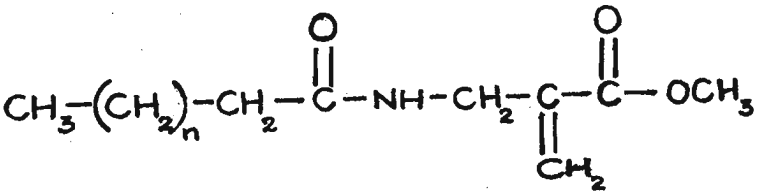
1.1.5 Biological significance of Marine Toxins

A toxin is an entity that has an adverse physiological effect on a living organisms, even when applied in small quantity. They are animal secretions produced by discrete glands. Some of these marine toxins are used as defensive substances against predation. It has been suggested that toxins produced by corals or sponges have a protective action not only against predators, but also against larval forms of sessile animals.[17] Some of the organisms belonging to the phylum Cnidaria and phylum Polifera are toxic to man. Specially Cnidarian organisms like soft corals contain venomous organs called nematocysts. Out of 9000 species of Cnidarians, about 70 species produce toxins in their nematocysts. The most of the nematocysts are located on the tentacles. They have a oval-shaped capsule, which encloses a coiled thread tube. When stimulated, the nematocysts release the coiled thread and tips of the thread tubes penetrate the epithelium of the victim. The venom in the capsule is then injected into the tissue of the victim through the tabule.[17]

Marine alga too contain toxins. The algal toxin has a drastic effect on the marine ecosystem, killing large numbers of macro-algae, invertebrates, and fish.[32] Professor Rutger Rosenberg reported a very strong toxic compound from the dominant alga Chrysochromulina polylepis. Water containing C. polylepis has been shown to be highly toxic to Artemia spp; a 24 hr. LC 50 was obtained at cell concentrations as low as 0.2 million per litre. This inhibited fertilization and larval development in blue mussels and Ciona intestinalis at about 1.7 million per litre cell concentration.

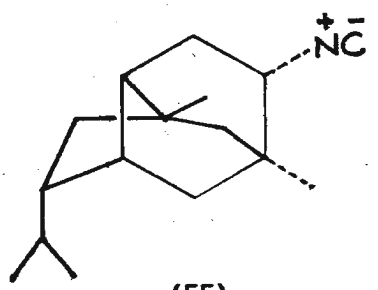
Among the toxins, isolated from marine fish, tetrodotoxin (**53**) play an important role in the field of pharmacology. In 1909, Tahara first isolated[33] a crude extract of this toxin and later several research groups modified the isolation procedure.[17] Strong pharmacological activities such as permeability of sodium ions in the nerve membranes, paralysis of peripheral nerves led to extensive research on this compound to develop it as a drug.

The investigation of toxic compounds from marine sponges has also led to the isolation of interesting toxins. In 1973, Kashman et al. isolated N-acyl-2-methylene-β-alanine methyl ester (**54**) with lethal dose 2-4mg/25g from the sponge Fasciospongia cavernosa.[34]



(54)

The sesquiterpene toxin 9-isocyanopupukeanane (**55**) has been isolated from the marine sponge Hymeniacidon sp. and the nudibranch Phyllidia varicosa which feeds on Hymeniacidon.[35]



(55)

Discovery of metabolites showing various forms of biological activity such as anti-bacterial, anti-fungal, anti-fertility, cytotoxic and anti-tumor activity from marine organisms has led to extensive research programmes aimed at isolation and characterization of biologically active secondary metabolites from marine organisms.

2. ISOLATION AND STRUCTURE ELUCIDATION

2.1 Studies on Sinularia abrupta (Kalkudah collections)

2.1.1 Collection and extraction

Fresh specimens of the soft coral Sinularia abrupta were collected at a depth 2.5 -5 meters from the coastal waters at Kalkudah in the eastern coast of Sri Lanka by SCUBA diving. The fresh organism was immediately cut into small pieces (approximate size 3cm x 3cm x 3cm) and extracted with methanol-methylene chloride (1 : 1) for four weeks at room temperature. The solvent was decanted off and concentrated under reduced pressure to obtain a dark brown residue (4.00g).

2.1.2 Initial fractionation

The residue (4.00g) was dissolved in aqueous methanol and extracted with light petroleum, methylene chloride and ethyl acetate respectively. The methylene chloride extract was dried over anhydrous Na_2SO_4 and the solvent was evaporated under reduced pressure to obtain a dark brown solid (3.41g). This was subjected to vaccum liquid chromatography on t.l.c. grade silica gel (500g)

in methylene chloride-methanol (100:0 ⟶ 75:25).

Thirteen fractions were collected.

Fraction 1	106.7mg
Fraction 2	20.5mg
Fraction 3	82.2mg
Fraction 4	97.2mg
Fraction 5	51.8mg
Fraction 6	29.8mg
Fraction 7	30.2mg
Fraction 8	112.7mg
Fraction 9	109.8mg
Fraction 10	1.282g
Fraction 11	1.07g
Fraction 12	133.5mg
Fraction 13	15.2mg

Fraction 11 (1.07g) was subjected to further chromatographic separation on silica gel column (60-120 mesh, 15g) in methylene chloride-methanol (100:0 ⟶ 95:5) to obtain five fractions.

Fraction i	10.7mg
Fraction ii	12.0mg
Fraction iii	30.2mg
Fraction iv	686.2mg
Fraction v	150.3mg

2.1.3 Isolation and characterization of furanocembranoid (**56**)

Fraction (iv) yielded a crystalline residue (686mg). It was chromatographed on a bio-sil A column (200-400 mesh, 14g) in ethyl acetate-hexane (10:90 ⟶ 40:60). Fractions eluted with ethyl acetate-hexane (40:60) yielded a crystalline compound, t.l.c. analysis of which in hexane-ethylacetate (1:1) showed the presence of one major component which gave a red spot of R_f 0.48 with vanilline/Conc. H_2SO_4. It was further purified by repeated recrystallization from ethanol to obtain white needles of the furanocembranoid diterpene(**56**)(50mg); m.p. 169-172°C, $[\alpha]_D^{26.5}$ −11 (C = 2, $CHCl_3$), elemental analysis C62.56, H6.80, O30.64, for $C_{22}H_{28}O_8$: C 62.85, H 6.71, O 30.44; uv λ max (MeOH); 213(5400), 243(2700) nm; ir ν max (film); 3685, 3620, 2960, 1715, 1520, 1220, 1050, 930, 880,850 cm^{-1}.

The IR spectral bands at 3620 cm^{-1} and 3685 cm^{-1} together with two broad singlets at δ 2.71 and 4.34 ppm which showed saturation transfer (i.e. irradiation of H_2O peak at δ 1.64 ppm reduced the intensity of hydroxyl peaks) from the water peak in the ^{1}H-NMR spectrum indicated that the compound is a diol. Of the two hydroxyl groups, one is secondary as indicated by the irradiation of the OH absorption at δ 2.73 (brs) which caused sharpening of the 1H broad singlet at δ 4.64 to a doublet (J=0.8 Hz) and by the ^{13}C-NMR doublet at δ 74.35 ppm. The irradiation of the second OH peak at 4.34 ppm resulted in a clear sharpening of the methyl group resonance (brs) at δ 1.24 ppm. This shows

that the second OH group is close to a methyl group and it is a tertiary hydroxyl function as indicated by a ^{13}C singlet at δ 73.64 ppm.

The ^{13}C-NMR signals at δ 163.96 (s), 167.22(s), 51.17 (q) and 51.43(q) ppm and a broad absorption in the IR spectrum at 1715 cm^{-1} together with two-OCH_3 singlets in the ^{1}H-NMR spectrum at 3.75 and 3.80 ppm indicated the presence of two conjugated methyl ester functions.

A prominent structural feature of (**56**) is the α,α' - dialkyl-β -furan carboxylate function. This is evident from the ^{13}C-NMR absorptions at δ 160.17 (s,C-3), 114.89 (s,C-4), 108.84(d,C-5) and 151.25(s,C-6) in the off resonance decoupled spectrum. These values are in good agreement with those reported for a similar function in the furanocembranolide, pukalide (**5**) with ^{13}C-NMR signals at δ 160.00(s,C-3), 113.90(s,C-4), 106.40(d,C-5) and 148.20 (s,C-6) ppm. The β'-proton of the furan ring in pukalide resonated at δ 6.33 ppm as a singlet. In compound (**56**), the β' -proton of the furan ring resonates at δ 6.61 ppm as a doublet due to small allylic coupling (0.8Hz) with C_7-H. Irradiation of the broad singlet at 4.64 ppm due to C_7-H caused the doublet at 6.61 ppm (1H, J=0.8Hz) to collapse to a singlet.

The presence of an isopropyl group in (**56**) was confirmed by the signals at δ 1.83 (brs) ppm for the allylic methyl and signals at δ 4.97 (t, J=1.5Hz) and 4.89 ppm (brs) for the terminal methylene protons. This is further evident from the IR absorption band at 930 cm^{-1}. All these signals are compatible with the corresponding signals for pukalide at δ 1.75 (3H,s) (allylic methyl), δ 5.20 (brs) and 4.91 (brs) ppm (olefinic protons) in the ^{1}H-NMR spectrum and the IR absorption bond at 890 cm^{-1}.

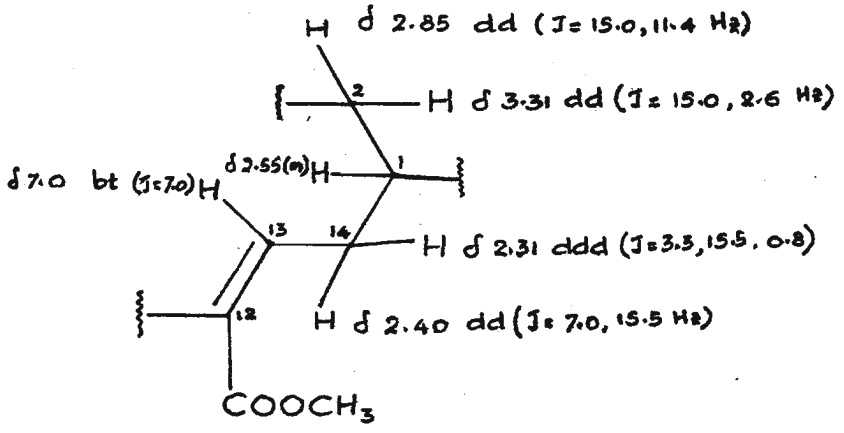
A broad triplet at δ 7.00 ppm in the ^{1}H-NMR spectrum was found to be due to the olefinic proton of a α,β -disubstituted methyl acrylate unit. Irradiation of this proton causes the dd at 2.40 ppm (J=7.0, 15.5Hz) to collapse to a doublet (J=15.5Hz), while irradiation at δ 2.31 (ddd) ppm collapses the broad triplet at δ 7.00 ppm to a broad singlet indicating that β -olefinic proton is adjacent to a methylene with hydrogens at δ 2.31 ppm and 2.40 ppm.

In addition, this irradiation simplified the multiplet at δ 2.55 ppm and caused the broad triplet at δ 7.0 ppm to collapse to a broad singlet showing that the CH proton which resonates at δ 2.55 ppm is adjacent to the methylene protons on C-14 atom.

This was confirmed by the irradiation of the multiplet at δ 2.55 ppm, which caused the simplification of the ddd at δ 2.31 to a dd.

Irradiation at δ 2.55 ppm caused the two protones on C-2 which resonate at δ 2.85 dd (J=11.4, 15.0Hz) and δ 3.31 dd (J=2.6, 15.0Hz) to collapse to two doublets indicating the presence of an adjacent methylene unit.

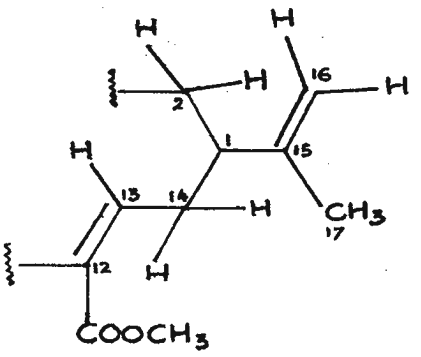
These irradiations are completely consistant with the presence in the molecule of the unit I.



I

The broad singlet at δ 4.89 ppm and triplet at δ 4.97 ppm (1.5Hz) due to the terminal olefinic protons of the isopropyl group sharpened upon the irradiation of the multiplet at δ 2.55 ppm. This indicates that isopropyl group is attached to the C-1 carbon atom. The irradiation of the methyl proton singlet at δ 1.83 ppm too caused sharpening of the signals due to the terminal olefinic protons.

Hence the partial structure II can be assigned for the molecule.



II

Further, irradiation at δ 2.55 ppm (1H,m) caused the two double dublets at δ 3.31 (J=2.6, 15.0Hz) and δ 2.85 (J=11.4, 15.0Hz) to collapse into two dublets of coupling constant 15.0Hz each, whereas irradiation at δ 3.31 (J=2.6, 15.0Hz) simplified the multiplet at δ 2.55 ppm and the double dublet at δ 2.85 ppm. This is evidence for the presence of a methylene group adjacent to C-1 carbon atom. This is supported by the ^{13}C-NMR absorption at δ 30.77 (dd) ppm of C-2 carbon atom.

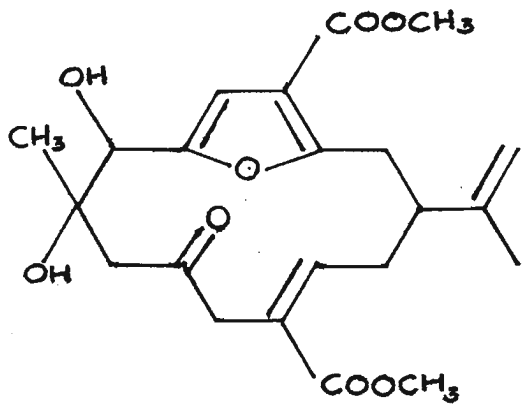
Upon irradiation of the ^{1}H-NMR signal at δ 2.54 ppm (C-9,d) the doblet at 3.15 collapsed into a singlet.

The SP^2 carbon singlet at δ 209.73 ppm in the ^{13}C-NMR spectrum belongs to a saturated ketone. The assignment of the carbon signals, especially those of the four methylene groups at δ 46.53,

42.39, 30.77 and 30.68 ppm was made through ^{13}C x ^{1}H correlations derived from a series of off-resonance decoupled spectra. In view of the possible flexibility of the 14-membered ring, it is difficult to suggest a relative stereochemistry for the three chiral centres.

Of the nine degrees of unsaturation of the molecule, seven can be assigned to two ester carbonyls, 1 ketone & 4 double bonds. The other two degrees of unsaturation are due to two rings; a furan and a 14-membered cembrane ring.

Thus, on the basis of the spectral data, structure (**56**) can be assigned to this new furanocembranoid diterpene.



(56)

^{1}H-nmr (300 MHz, $CDCl_3$): δ 1.24 (3H, brs, C_{10}-H_3), 1.83 (3H, brs, C_{17}-H_3), 2.31 (1H, ddd, J=15.5, 8.0, 3.3 Hz, C_{14}-H), 2.40 (1H,dd, J=15.5, 7.0Hz, C_{14}-H), 2.54 (1H, d, J=18.0 Hz, C_9-H), 2.55 (1H, m,

C_1-H), 2.73 (1H, brs, C_7-OH), 2.85 (1H, dd, J=15.0, 11.4 Hz, C_2-H) 3.15 (1H, d, J=18.0 Hz, C_9-H), 3.31 (1H, dd, J=15.0, 2.6 Hz, C_2-H) 3.41 (1H, brd, J=12.2 Hz C_{11}-H), 3.51 (1H, d, J=12.2 Hz, C_{11}-H), 3.75 (3H, s, C_{21}-H_3) 3.80 (3H, s, C_{22}-H_3), 4.34 (1H, brs, C_8-OH), 4.89 (1H, brs, C_{16}-H), 4.97 (1H, t, J=1.5 Hz, C_{16}-H), 6.61 (1H, d, J=0.8 Hz, C_5-H), 6.64 (1H, brs, C_7-H), 7.00 (1H, brt, J=7.0 Hz, C_{13}-H) : ^{13}C n.m.r. (75.5 MHz, $CDCl_3$) : δ 21.07 (q), C-17, 24.44, (q), C-19, 30.68, (t), C-14, 30.77 (t), C-2, 42.39 (t), C-9, 43.36 (d), C-1, 46.53 (t), C-11, 51.43 (q), C-21, 52.17 (q), C-22, 74.35 (d), C-7, 73.64 (s), C-8, 108.84 (d), C-5, 111.97 (t), C-16, 114.89 (s), C-4, 125.57 (s), C-12, 143.56 (d), C-13, 145.63 (s), C-15, 151.25 (s), C-6, 160.17 (s), C-3, 163.96 (s), C-18, 167.22 (s), C-20, 209.73 (s), C-10 : Ms m/z (%, C.I) 421 (M + 1) (3), 403 (100), 371 (42), 353 (13), 339 (9).

2.1.4 Isolation and characterization of furanocembranoid (**57**)

Fraction (v) (150 mg) from section 2.1.2 was subjected to further chromatographic separation on silica gel (70-230 mesh, 150 g) in methylene chloride-methanol (100;0 ⟶ 90:10). Five fractions were

obtained.

Fraction 1 - 26.5 mg

Fraction 2 - 30.1 mg

Fraction 3 - 20.0 mg

Fraction 4 - 27.5 mg

Fraction 5 - 10.3 mg

Fraction (2) (30 mg) on t.l.c. analysis (5% MeOH in CH_2Cl_2) showed the presence of one major component which gave a dark red spot with vanilline/conc. H_2SO_4. This was further purified by chromatography on silica gel in ethyl acetate - light petroleum of increasing polarity to obtain 4 fractions.

Fraction i - 2.0 mg

Fraction ii - 12.0 mg

Fraction iii - 5.1 mg

Fraction iv - 4.2 mg

The cyrstalline fraction ii (12.0 mg) was further purified by high performance liquid chromatography on silica gel (flow rate = 3ml/min) in light petroleum - ethyl acetate (1:1) to obtain the furanocembranoid diterpene **(57)** (8.0 mg) as a white crystalline solid, m.p. 182-185^{o}C; IR (film) ν max: 3520, 2950, 1725, 1650, 1625 cm^{-1}; ^{1}H-NMR (300 MHz, $CDCl_3$) : δ 1.35 (3H, brs, J=0.9 Hz, C_{19} -H_3), 1.60 (3H, brs, J=0.5 Hz, C_{17}-H_3), 1.75 (1H, m, C_1-H), 2.05 (1H,dd, J=5.1, 9.5 Hz, C_2-H), 2.20 (2H, m, C_{14}-H_2), 2.55 (1H, dd, J=6.4, 8.2 Hz, C_2-H), 2.70 (1H, dd, J=1.4, 1.8 Hz, C_9-H), 3.08 (3H, s, C_{23}-OCH_3), 3.23 (1H, s, C_9-H),

3.33 (1H, d, J=17.3 Hz, C_{11}-H_a), 3.53 (1H, d, J=17.3 Hz, C_{11}-H_b), 3.73 (3H, s, C_{21}-H_3), 3.78 (3H, s, C_{22}-H_3), 4.28 (1H, s, C_8-OH), 4.58 (1H, brs, C_{16}-H), 4.73 (1H, t, J=1.4 Hz, C_{16}-H), 5.17 (1H, brs, J=1.4Hz, C_7-H), 6.95 (1H, s, C_5-H), 7.00 (1H, m, C_{13}-H); ^{13}C-NMR (75 MHz, $CDCl_3$): δ 19.57 (q) C-17, 27.38 (q) C-19, 33.20 (t) C-14, 39.77 (t) C-2, 40.97 (t) C-9, 41.48 (d), C-1, 50.03 (q), C-22, 51.47 (t), C-11, 51.91 (q), C-21, 52.20 (q), C-23 70.76 (s), C-8, 112.87 (t), C-16, 115.94 (s), C-4, 117.03 (d), C-5, 128.13 (s), C-12, 131.26 (s), C-15, 139.01 (d), C-7, 143.38 (d), C-13, 145.69 (s), C-3, 150.13 (s), C-6, 162.06 (s), C-18, 167.37 (s), C-20, 210.77 (s), C-10 ; MS m/z: 434 (M^+)

The IR absorption band at 3520 cm^{-1} indicated that the compound has a hydroxyl group.

The ^{13}C-NMR signals at δ 162.0 (s), 167.40 (s), 51.91 (q) and 52.20 (q) ppm and a strong absorption in the IR spectrum at 1725 cm^{-1} together with two -OCH_3 singlets in the ^{1}H-NMR spectrum at δ 3.73 and 3.78 ppm indicated the presence of two conjugated methyl ether functions.

The presence of an α,α'-dialkyl furanocarboxylate function in the compound was confirmed by the ^{13}C-NMR absorptions at δ 145.69 (s, C-3), 131.26 (s, C-4), 117.03 (d, C-5) and 150.13 (s, C-6). The proton of the furan ring of this compound resonates at δ 6.95 ppm as a singlet.

The presence of an isopropyl group was confirmed by the signals at δ 1.60 ppm (brs, J=0.5Hz) for the allylic methyl and peaks at δ 4.58 (brs) and δ 4.73 ppm (t, J=1.4 Hz) for the terminal olefinic protons.

The broad singlet at δ 4.58 ppm and triplet at 4.73 ppm (J=1.4 Hz) due to the terminal olefinic protons of the isopropyl group sharpened upon the irradiation of the multiplet at δ 1.75 ppm. This indicates that isopropyl group is attached to the C-1 carbon atom. In addition, the irradiation of the olefinic proton at δ 4.58 ppm (brs) causes the triplet at δ 4.73 ppm to collapse to a doublet and increases the peak at δ 1.60 ppm, while irradiation at δ 4.73 ppm (t, J=1, 4Hz) resulted in a clear sharpening of the peaks at δ 4.58 and 1.60 ppm.

The irradiation of the broad singlet at δ 5.17 ppm (C_9-H) causes the dd at δ 2.70 (C_9-H, J=1.4, 1.8 Hz) to collapse to two singlets. The broad singlet at δ 5.17 ppm (J=1.4 Hz) sharpened upon the irradiation of the dd at δ 2.70 ppm. The difference in chemical shifts of these two protons may be due to the effect of the OH group. The singlet at δ 210.77 ppm in the ^{13}C-NMR spectrum is due to the saturated ketone.

The methylene protons at δ 3.33 and δ 3.53 ppm (C_{11}-2H) suggests a $-(CH)_2-$ group between C-$\overset{O}{\overset{\|}{C}}$- and C=C double bond.

In the ^{1}H-NMR spectrum, the olefinic proton of the α,β-disubstituted methyl acrylate unit resonates at δ 7.00 ppm (1H) as a multiplet. The irradiation of this multiplet caused the simplification of the

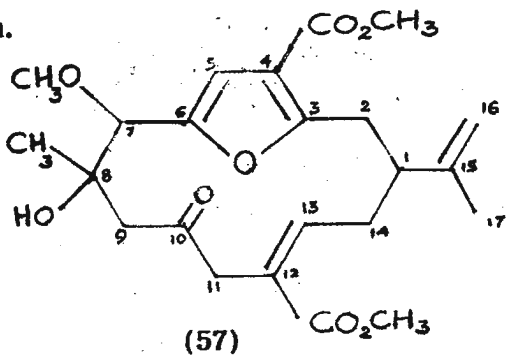
multiplet at δ 2.20 ppm, while irradiation at 2.20 ppm caused the simplification of multiplet at δ 7.00 ppm. These irradiations indicate that the olefinic proton is adjacent to the methylene protons at δ 2.20 ppm.

In addition, the irradiation of the proton at δ 1.75 ppm simplified the multiplet at δ 2.20 ppm indicating that the C_1-H proton at δ 1.75 ppm is adjacent to the methylene protons on C-14 atom.

Further, irradiation of the proton at δ 1.75 ppm caused the two protons on C-2 which resonate at δ 2.05 (dd, J=5.1, 9.5 Hz) ppm and δ 2.55 (dd, J=6.4, 8.2 Hz) to collapse to two doublets indicating the presence of an adjacent methylene unit.

Irradiation of the dd at δ 3.33 ppm (C_{11}-H_a) simplified the dd at δ 3.53 ppm (C_{11}-H_b) while irradiation of the dd at δ 3.53 ppm simplified the dd at δ 3.33 ppm.

The DEPT spectrum of this compound showed a tertiary carbon atom at δ 70.76 ppm.



(57)

The irradiation of the peaks at δ 4.28 ppm (s, 1H) and 1.35 ppm (s,3H) ppm respectively in the ^{1}H-NMR spectrum indicated that they

are mutually coupled to each other.

The molecular ion in the mass spectrum at 434 indicated that the molecular formula was $C_{23}O_8H_{30}$. There are therefore 9 degrees of unsaturation; two ester carbonyls, one ketone, four double bonds, one furan ring and one 14-membered cembrane ring.

On the basis of these spectral data, structure (**57**) was assigned to this cembranoid diterpene.

2.2 Studies on Sinularia crispa (Unawatuna collection)

2.2.1 Collection and Extraction

The alcyonacean soft coral Sinularia crispa was collected at a depth of 5-10 meters from the coastal waters at Unawatuna in the southern coast of Sri Lanka in 1986. The fresh specimens were cut into small pieces (approximate size 3cm x 3cm x 3cm) and extracted with methylene chloride : methanol (1 : 1) for two weeks at room temperature. The solvent was decanted off and the specimens were extracted again three times with the same solvent system. The combined methylene chloride - methanol (1 :1) extract was concentrated under reduced pressure to give a dark residue (62.2g).

2.2.2 Isolation and characterisation of 1,O-octadecyl-sn-glycerol (batyl alcohol) (**58**)

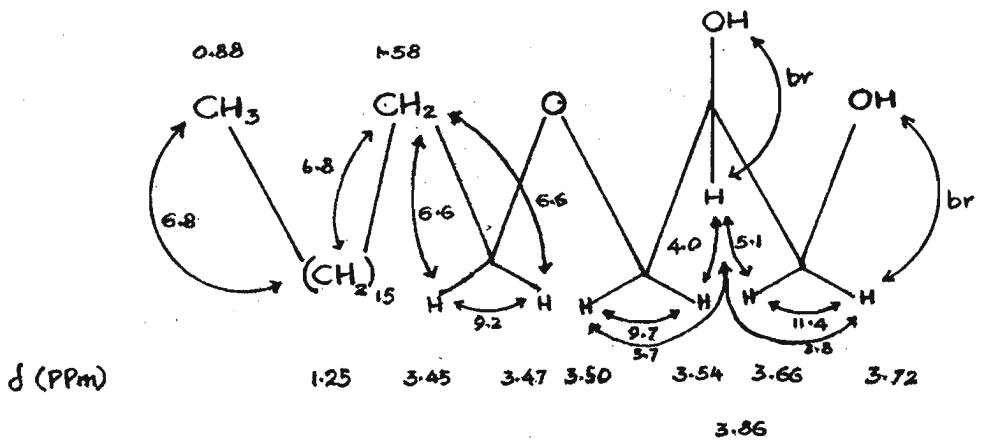
The crude extract (62.2g) was dissolved in aqueous methanol and

partitioned into light petroleum and chloroform respectively. The chloroform extract (15.2g) was subjected to chromatographic seperation on a Sephadex LH-20 column in hexane-methylene chloride-ethyl acetate-water (10 : 10 : 1 : 1) to obtain nine fractions.

The fifth fraction was subjected to medium pressure liquid chromatography on a C-18 reverse phase column. The eluent was changed linearly from water-methanol (30:70) to pure methanol during a 4-hour period. Fractions eluted with water-methanol (10:90) yielded a white solid (65.2 mg) which on recrystallisation from methanol gave white needles (59.0 mg), m.p. 69°C, $[\alpha]_D$- 1.2 (C=44.9, $CHCl_3$); ^{1}H-NMR (300 MHz, $CDCl_3$): δ 0.88 (3H,t, J=6.80Hz, C_1-H_3), 1.25 (30H, brs, $(-CH_2-)_{15}$-H_{30}) 1.58 (2H,q, J=6.80Hz, C_{17}-H_2), 1.61 (2H, brs, H_2O), 2.18 (1H, brs, C_{21} OH), 2.61 (1H, brs, C_{20}-OH), 3.45(1H, dt, J=9.20, 6.60 Hz, C_{18}-H), 3.47 (1H, dt, J=9.20, 6.60 Hz C_{18}-H), 3.50 (1H, dd, J=9.70, 5.70 Hz, C_{19}-H), 3.54 (1H, dd, J=9.70, 4.00 Hz, C_{19}-H), 3.66 (1H, brdd, C_{21}-H), 3.72 (1H, brd, C_{21}-H), 3.86 (1H, brs, C_{20}-H).

The IR absorption at 3420 cm^{-1} indicated the presence of hydroxyl group in the molecule. The broad singlets in the ^{1}H-NMR spectrum of this compound at δ 2.61 and δ 2.18 ppm which showed saturation transrer from the water peak (i.e. irradiation of water peak at δ 1.61 ppm led to the disappearance of the -OH peaks) indicated that the compound is a diol. Of the two hydroxyl groups, one is secondary as indicated by the irradiation of the OH absorption at δ 2.61 ppm, which caused sharpening of the broad singlet at δ 3.86 ppm to a ddt (J=5.7, 5.1 4.0 Hz).

Further, this irradiation caused the disappearance of the second OH absorption at δ 2.18 (brs) indicating that it is in close proximity to the secondary OH group and also the disappearance of the H_2O peak at δ 1.61 (br) ppm. The second OH group is primary, since the irradiation of this signal at δ 2.18 ppm resulted in a clear sharpening of the methylene protons at δ 3.72 (brd), δ 3.66 (brdd) ppm to two double doublets (J=11.4, 3.8 Hz) and (J=11.4, 5.1 Hz), disappearance of secondary OH absorption at δ 2.61 ppm and H_2O absorption at δ 1.61 ppm.



(58)

Irradiation of the proton at δ 3.86 (br) ppm which is attached to the carbon carrying the secondary hydroxyl group resulted in a clear sharpening of the two broad signals at δ 3.66 ppm and 3.72 ppm into two broad doublets each with a coupling constant of 11.4 Hz, and the two double doublets which represent an ABX systems at 3.54 ppm (J=9.7, 4.0 Hz) and δ 3.50 ppm (J=9.7, 5.7 Hz) into two doublets each with a coupling constant 9.7 Hz.

Irradiation of the signal corresponding to 30 protons $(CH_2)_{15}$ which resonates at δ 1.25 ppm (brs) causes the second order triplet at δ 0.88 ppm to collapse to a singlet and the multiplet at δ 1.58 (J=6.8 Hz)ppm to a triplet (J=7.0 Hz).

Irradiation at δ 1.58 ppm (q) causes the two dt's in the ABX_2system which resonates at δ 3.45 (J=9.2, 6.6 Hz) and δ 3.47 (J=9.2, 6.6 Hz) ppm to collapse to two doublets with the coupling constant 9.2 Hz each. The couplings among these protons were clearly seen in the homocorrelated 2D-^{1}H-NMR (COSY) spectrum.

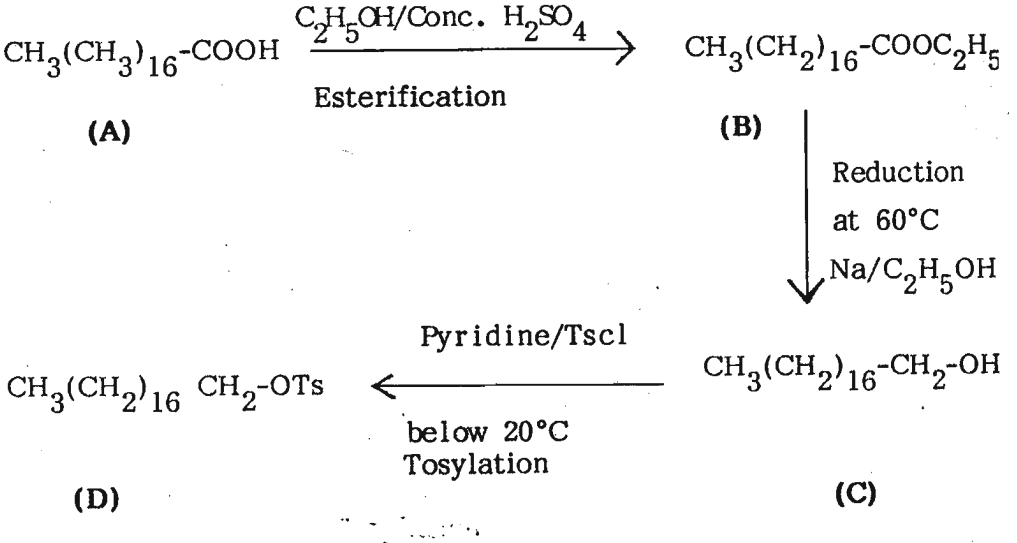
The molecular ion (M^+ = 344) in the mass spectrum was consistent with the molecular formula $C_{21}H_{44}O_3$. On the basis of these spectral data, the compound was identified as 1,O-octadecyl-sn-glycerol (batyl alcohol)(58)

It was intended to confirm the structure of this naturally occuring fatty acid derived glyceryl ether which showed strong antibacterial activity against Pseudomonas sp. by its synthesis, starting from glycerol and stearic acid.

2.2.3 Synthesis of 1-O-octadecyl-sn-glycerol (58)

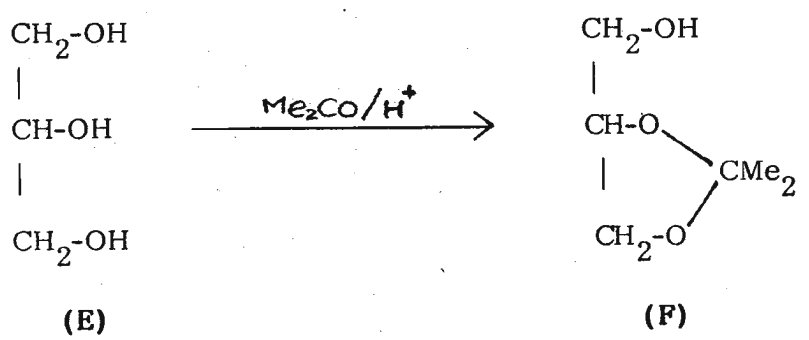
The esterification of stearic acid (A) with 99.8% ethyl alcohol in the presence of a few drops of conc.H_2SO_4 acid yielded ethyl stearate (B). Reduction of ethyl stearate with sodium/ethanol at 60°C[36] followed by purification through chromatographic separation on bio-sil gave the alcohol (C). The absence of carbonyl absorption at 1725 cm^{-1} in the

IR spectrum of the alcohol confirmed that the reduction was complete. The tosylation[37] of the alcohol with p-toluenesulfonyl chloride in pyridine at a temperature below 20°C yielded n-octadecyl p-toluenesulfonate (D) which was purified by recrystallization from light petroleum. (Scheme 1).

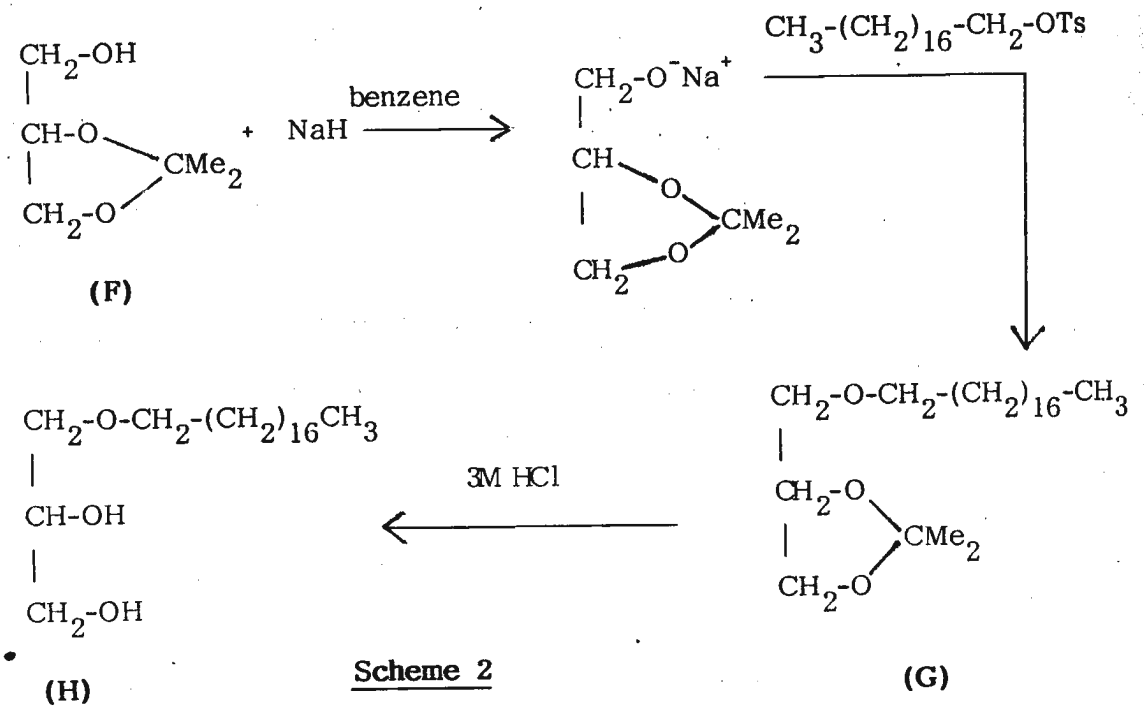


Scheme 1

Reaction of distilled, dry glycerol (E) with 95% acetone in the presence of the acid catalyst p-toluenesulfonic acid monohydrate yielded isopropylideneglycerol (2,2-dimethyl-1,3-dioxolane-4-methanol) (F).[38]



Isopropylideneglycerol (6.0 mg) was treated with NaH(1 mg) in the presence of dry benzene (3.0 ml) and n-octadeyl p-toluenesulfonate (20 mg) was added to this mixture and allowed to react over night. The product (G) was treated with 3M HCl (3 x 3 ml) in order to remove the protecting group and then neutralized with 10M NaOH (10ml). This was extracted with chloroform (3 x 5 ml) and concentrated under reduced pressure to obtain batyl alcohol (H). (Scheme 2)



Scheme 2

These reactions were monitored by TLC analysis as shown in figure 1 and 2.

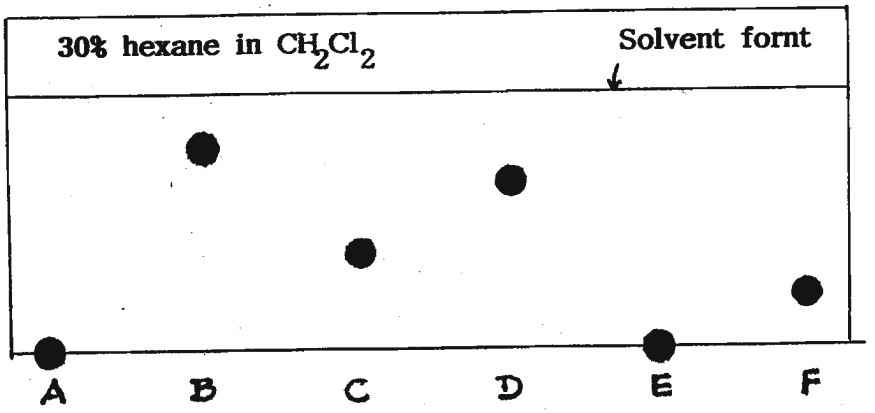


Figure 1

A - Stearic Acid

B - Ethyl Stearate

C - Alcohol (C_{18})

D - Tosylated Alcohol

E - Glycerol

F - Isopropylideneglycerol

H - Synthetic product

I - Natural Product (from Sinularia crispa)

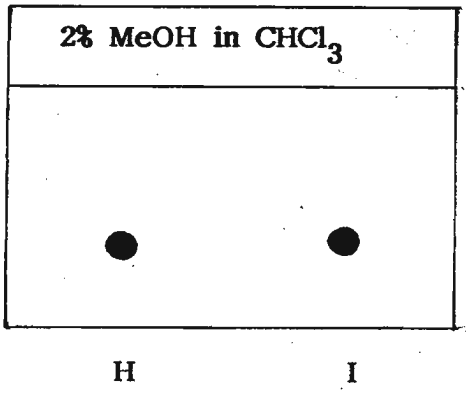


Figure 2

2.3 Studies on Sinularia abrupta (Unawatuna collection)

2.3.1 Collection and Extraction

Fresh specimens of the soft coral, Sinularia abrupta was collected at a depth of 5-10 meters from the coastal waters at Unawatuna in the southern coast of Sri Lanka in February, 1986. The fresh specimens were cut into small pieces (approximate size 3cm x 3cm x 3cm) and extracted with methylene chloride-methanol (1:1) for five weeks at room temperature. The solvent was decented off and concentrated under reduced pressure. The aqueous residue was partitioned into light petroleum and methylene chloride respectively.

2.3.2 Isolation and Characterisation of diacyl glyceryl ether (**59**)

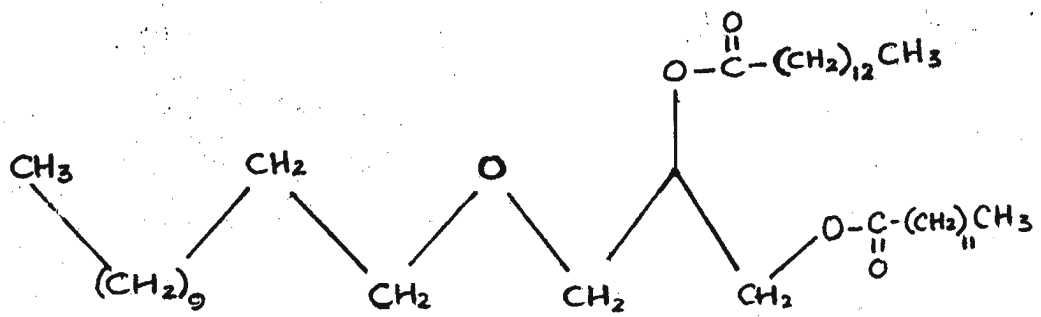
The methylene chloride solubles were concentrated under reduced pressure to give a brown residue (3.21 g). The residue (3.21 g) was subjected to vaccum liquid chromatography on t.l.c. grade silica gel (15.0 g) in hexane - CH_2Cl_2 (100:0 ⟶ 20:80).

Fractions eluted with hexane - CH_2Cl_2 (80:20) yielded a white crystalline solid (240 mg). It was further purified by recrystallization from methanol to obtain white needles of the diacyl glyceryl ether (**59**) (190 mg); m.p. 52°C, $[\alpha]_D$-17.4 (C=4.6, $CHCl_3$); $C_{42}H_{82}O_5$: Ms m/z: 653 (M^+-C_2H_6); ^{1}H-NMR (300 MHz, $CDCl_3$): δ 0.88 (3H, t,C_1-H_3), 1.26 (18H, brs, (C_2-C_{10})-H_{18}) 1.58 (2H, m.C_{11}-H_2), 3.42 (1H, dt, C_{12}-H), 3.43 (1H, dt, C_{12}-H) 4.15 (1H, dd, C_{13}-H), 4.32 (1H, dd, C_{13}-H), 5.20 (1H, ddt, C_{14}-H), 3.53 (2H, brd, C_{15}-H_2), 2.29 (2H, t,C_{16}-H_2), 2.32 (2H, t, C_{22}-H_2).

The IR spectrum of this compound showed an absorption at 1720 cm^{-1} indicating that the compound has a ester carbonyl function.

Most of the signals in the ^{1}H-NMR spectrum of this compound closely resembled those of the batyl alcohol (**58**). In the ^{1}H-NMR spectrum, the broad triplet at δ 0.88 ppm is attributed to terminal methyl groups. By compairing with the ^{1}H-NMR of (**58**) the signal at δ 1.26 ppm (brs) can be assigned to long chain methylene protons. The two protons α-to the ether oxygen of the long chain alcohol moiety resonated at δ 3.42 and δ 3.43 ppm as triplets representing an ABX_2 system as

described for the batyl alcohol (**58**). The two clear double doublets which resonated at δ 4.15 and δ 4.32 ppm were attributed to the methylene protons of glycerol attached to the ether functionality. Irradiation of the signal at δ 4.15 ppm resulted in a simplification of the peak at δ 5.20 ppm (ddt). This irradiation caused the peak at 4.32 ppm (dd) to collapse into a doublet (J=9.0 Hz) Irradiation of the peak at δ 5.20 ppm caused the two double doublets at δ 4.15 and δ 4.32 ppm to collapse to two doublets (J=8.2 each) and the doublet at δ 3.53 ppm to a sharp singlet.



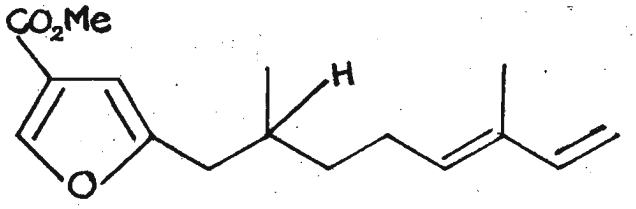
(59)

The two sets of methylene protons α -to the ester cabonyl in the two diacyl units resonated at δ 2.29 and δ 2.32 ppm as two triplets. In the ^{13}C-NMR spectrum, the two singlets at δ 173.1 and δ 173.4 ppm are attributed to the carbonyl carbons of the two acyl units.

On the basis of these spectral data, structure (**59**) was assigned to this diacyl glyceryl ether.

2.3.3 Isolation and Characterization of Methyl (5'E) -5-(2',6'-dimethylocta-5',7'-dienyl) furan-3-carboxylate (13)

Fractions eluted with hexane- CH_2Cl_2 (95:5) from section 2.3.2, was subjected to high performance liquid chromatography on silica gel in hexane to obtain the furanosesquiterpene (**13**).



(13)

The IR absorption band at 1732 cm^{-1} together with ^{13}C singlet at δ 162 ppm and ^{1}H-NMR singlet at δ 3.83 ppm indicated that the compound has a methyl ester group.

The ^{13}C-NMR signals at δ 146.0 (d), 114.2 (s), 107.0 9d) and 159.0 (s) ppm and signals at δ 7.9 ppm (brs) & 6.34 ppm (brs) in the ^{1}H-NMR spectrum indicated the presence of an α- subsitituted furan ring. Irradiation of the broad singlet at δ 6.34 ppm caused sharpening of the broad singlet at δ 7.9 ppm indicating the W coupling of the two protons in the furan ring.

Irradiation of the dd at δ 2.60 ppm in the ^{1}H-NMR spectrum simplified the multiplet at δ 1.83 ppm and caused the dd at δ 2.46 ppm to

collapse to a doublet while irradiation of the dd at δ 2.46 ppm simplified the multiplet at δ 1.83 ppm and caused the dd at δ 2.60 ppm to collapse to a doublet. This indicates that the two signals at δ 2.46 ppm and δ 2.60 ppm are due to methylene proton on 1'-carbon atom and the proton which resonated at δ 1.83 ppm is adjacent to the 1'-carbon atom. Irradiation of the methyl signal at δ 0.93 ppm (d) simplified the multiplet at δ 1.83 ppm while irradiation of the multiplet at δ 1.83 ppm caused the doublet at δ 0.93 ppm to collapse to a broad singlet indicating the presence of a methyl group on 2'-carbon atom. Further the irradiation of the multiplet at δ 1.83 ppm simplified the two multiplets at δ 1.25 ppm and δ 1.40 ppm. This indicated that the two protons which resonated at δ 1.25 ppm and 1.40 ppm are attached to the 3' -carbon atom.

The irradiation of the methylene proton multipled at δ 2.18 ppm caused the simplification of the two multiplets at δ 1.25 ppm and δ 1.40 ppm, thus establishing the location of the methylene protons resonating at 2.18 ppm at 3' -carbon atom.

Further this irradiation caused the broad triplet at δ 5.45 ppm (J=17.34 Hz) to collapse to a singlet. This indicates that the olefinic proton in adjacent to methylene protons on 4' -carbon atom at 2.18 ppm. This was confirmed by the doublet at δ 134 ppm in the ^{13}C-NMR spectrum.

In the DEPT spectrum, the 6' -carbon atom, which resonated at δ 135 ppm indicated that it was a quartery carbon atom. The presence of terminal olefinic group was confirmed by the ^{1}H-NMR signals at δ 5.11 ppm (d, J=17.3 Hz), 4.95 ppm (d, J=10.7 Hz) and δ 6.77 ppm (dd, J=17.3,

10.17 Hz). The doublets at δ 5.11 and δ 4.95 ppm collapsed to two singlets on irradiation of the olefinic proton on 7^{t}-carbon atom which resonated at δ 6.77 ppm as a doublet of a doublet.

The couplings among these protons on each carbon atom were clearly seen in the homocorrelated 2D-^{1}H-NMR (COSY) spectrum.

The mass spectrum of the compound with a molecular ion of m/z 262.2 gave a molecular formula of $C_{16}H_{22}O_3$. There are therefore 6 degrees of unsaturation; one ester conbonyl, four double bonds and one furan ring.

On the basis of these spectral data, structure (13) was assigned to this furano-sesquiterpene.

2.4 Studies on an unidentified sponge (Wellawatta collection)

2.4.1 Collection and Extraction

The ash coloured unidentified sponge was collected at a depth of 10-15 meters from the coastal waters at Wellawatta in the western coast of Sri Lanka by SCUBA diving in February 1986. The fresh specimens were immediately chopped into small pieces and stored in methylene chloride-methanol (1:1) at room temperature for four weeks. The solvent was decanted off and concentrated under reduced pressure. The aqueous residue showed anti-bacterial activity against Staphylococcus aureus.

The extract was dried by azeotropic distillation using a mixture of benzene and ethanol. The residue (3.0g) was dissolved in a mixture of water-methanol (10:90) and extracted with light petroleum (100 ml x 3). The composition of the aqueous layer was adjusted to water-methanol (20:80) and extracted again with chloroform.

The composition of the aqueous layer was adjusted to water-methanol (30:70) and extracted with ethyl acetate.

2.4.2 Activity directed fractionation of $CHCl_3$ extract

The chloroform extract (1.18 g) which showed antibacterial activity against Staphylococcus aureus was separated by column chromatography on silica gel (Kieselgel 60, 70-230 mesh, 100 g) in light petroleum-acetone (90:10 $\longrightarrow$ 60:40) to obtain five fractions.

Fraction 1	-	120.6mg
Fraction 2	-	25.6mg
Fraction 3	-	434.4mg
Fraction 4	-	34.5mg
Fraction 5	-	27.2mg

Fraction 3 and 5 showed anti bacterial activity against S. aureus.

2.4.3 Isolation and characterization of variabilin (47)

Fraction 3 (434.4mg) was further chromatographed on a bio-sil A (200-400 mesh, 75.0g) column in ethyl acetate-methylene chloride (2:98 ⟶ 20:80) to obtain compound (**47**) (125 mg) as a pale yellow oil, which gave a red spot on t.l.c. (acetone-light petroleum 3:7) at R_f=0.57 with vanilline/ conc. H_2SO_4.

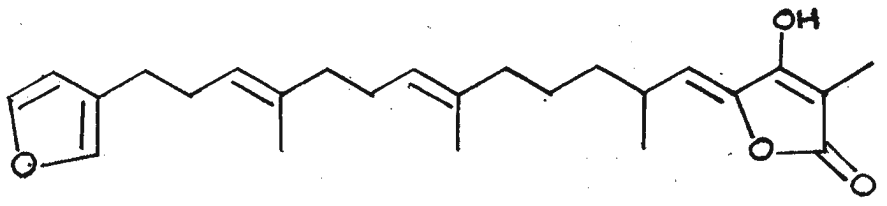
^{1}H-NMR (300.1 MHz, $CDCl_3$): δ 7.34 (1H, brs, C_1-H), 7.20 (1H brs, C_4 -H), 6.27 (1H, brs, C_2-H), 5.31 (1H, d, C_{20}-H), 5.15 (2H, m, C_{10}/ C_{12}-H_2) 2.80 (1H, brs, C_{22}-OH), 2.45 (2H, t,C_7-H_2), 2.25 (2H, t, C_{15}-H_2), 1.81 (3H, s, C_{25}-H_2), 1.65 (3H, s,C_9-H_3), 1.60 (3H, s, C_{14}-H_3), 1.07 (3H, d, C_{19}-H_3).

The characteristic pattern in the IR spectrum of this compound at 1025 cm^{-1}, 875 cm^{-1} and 770 cm^{-1} indicates the presence of a β-substituted furan ring in the molecule. This is confirmed by the three broad singlets at δ 7.34 (1H, C_1-H), 7.20 (1H, C_4-H) and 6.27 (1H, C_2-H) in the ^{1}H-NMR spectrum. These values are consistant with the corresponding values of variabilin and other related compounds with β -substituted furan ring such as fasciculatin, strobilinin and Ircinin.

In the UV spectrum, the λmax (MeOH) at 260 nm is characteristic of a

conjugated tetronic acid functionality. In the ^{1}H-NMR spectrum. the signals at δ 1.81 (3H, s, C_{25}-H_3) and 5.31 (1H, d, C_{20}-H) confirm the presence of tetronic acid moiety in the molecule.

By compairing with the ^{1}H-NMR signals of other related compounds, the structure of this compound which showed strong anti-bacterial activity against staphylococcus aureus was identified as variabilin (**47**) which D.J. Faulkner first isolated in 1973 from the sponge Ircinia variabilis.



(47)

Fraction 5 (27.2 mg) was separated by high performance liquid chromatography on microporasil column in hexane-ethyl acetate (3:2) to obtain the second anti-bacterial component (7 mg) as a pale yellow oil. This appeared to be related to variabilin according to the ^{1}H-NMR spectral data.

Screening for Anti-bacterial Activity

The bacteria used were Staphylococcus aureus, Pseudomonas sp. and E. coli. Twenty four hour cultures of the bacteria were used in the experiment. A tube each of Nutrient broth (appendix I) inoculated with a bacterium and incubated at 37°C for 24 hours.

Plates of Blood Agar base (appendix 2) were poured and once set lawned with 24 hr cultures of bacteria by placing two drops of the 24 hour broth culture of the bacterium on the agar plate and spreading this evenly on the agar surface with a sterile glass spreader.

Sterile filter paper discs of 0.7 cm diameter were used in the screening. Two discs per agar plate were dipped carefully under asceptic conditions in the extract in the appropriate solvent and allowed to dry for a few minutes in a sterile dish. The discs were transferred carefully with sterile forceps on to the lawned blood agar base dishes at two discs per extract per organism. Two discs per organism dipped in the neat solvent were used as a control. The plates were incubated at 37°C for 24 to 48 hours.

After 24 hrs, the clear areas round the discs which correspond to inhibition zones, where the growth of the bacterium has been inhibited by the extract on the filter disc, were measured.

Screening for Anti-fungal Activity

The extracts were spotted on a t.l.c. plate and developed using a suitable solvent system/ (Some pure compounds too were tested by this method.)

A culture of the fungus Cladosporium was grown in about 150 ml Czapek Dox solution (appendix 3) for seven days and the fungal mat was broken up and shaken throughly to get a spore suspension. This was filtered through two layers of muslin cloth to obtain a concentrated spore suspension in Czapek Dox solution. This was sprayed evenly on the dried t.l.c. plate using a spray gun and incubated in a moist chamber for 3 days. The Cladosporium growing on the t.l.c. plate as a black mold, white patches left on the plate where there were compounds with anti-fungal effects.

BIOASSAY RESULTS

Anti-bacterial Activity - Average Diameter (cm) of the Inhibitory Zone

Compound	Concentration mg/ml (CH_2CL_2)	Staphylococcus aureus	E. coli	Pseudomonas sp
(56)	5	-	-	
(57)	5	-	-	
(58)	4	-	-	0.80
(59)	6	1.00	-	0.85
(47)	13	1.60	-	

Anti-fungal Activity - Compound (13) showed anti-fungal activity against the fungus Cladosporium cladosporidae.

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APPENDIX 1

Nutrient broth

Beef extract	-	3.0g
Peptone	-	5.0g
Distilled water	-	1000 cm^3

Sterilized at 15 lb/sq inch for 15 minutes.

APPENDIX 2

Blood Agar base medium

Beef heart infusion	-	500g
Tryptose	-	10g
NaCl	-	5g
Agar	-	20g

pH 6.8

Sterilized at 15 lb/ sq. inch for 15 minutes.

APPENDIX 3

Czapek Dox Agar Solution

Sucrose	-	30g
$NaNO_3$	-	2.0g
K_2HPO_4	-	0.1g
KCl	-	0.5g
$MgSO_4.7H_2O$	-	0.5g
$FeSO_4$	-	trace
Agar	-	20g
Distilled water	-	1000 cm^3

pH 5.5

Sterilized at 15 lb/sq. inch for 15 minutes

APPENDIX 4

PUBLICATIONS

1. J.A. Chandrasiri, L.M.V. Tillekeratne, S.A. Deraniyagala and Ananda S. Amarasekara; Two Anti-bacterial Metabolites from a Sri Lankan Sponge. Proc. Sri Lanka Assoc. Advmt. Sci., 43 (1), 215 (1987)

2. J.A. Chandrasiri, L.M.V. Tillekeratne, S.A. Deraniyagala, M. Dai and John C. Coll; A new Furanoditerpenoid from a Sri Lankan Soft Coral. Proc. Sri Lanka Assoc. Advmt. Sci., 43 (1), 217 (1987)

3. J.A. Chandrasiri, L.M.V. Tillekeratne, S.A. Deraniyagala, A.S. Amarasekara, and Hugo E. Gottlieb; A new Furanocembranoid Di terpene from a Sri Lankan Soft Coral Sinularia abrupta. (in preparation)

4. J.A. Chandrasiri, L.M.V. Tillekeratne, S.A. Deraniyagala, A.S. Amarasekara and Hugo E. Gottlieb; Two Glyceryl Ether Derivatives from Sri Lankan Soft Coral Sinularia Spp. Proc. Sri Lanka Assoc. Advmt. Sci., 44 (1), 165 (1988)