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# RECENT RESEARCH IN MARINE NATURAL PRODUCTS FROM THE RED SEA

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Abstract - New metabolites isolated from soft corals and sponges collected in the Gulf of Eilat (The Red Sea) since 1974 are discussed.

## INTRODUCTION

It is the aim of this report to describe briefly part of the more interesting marine natural products isolated by us during the last few years. Our natural products research has been focused over the last years mainly on the chemistry of secondary metabolites from soft corals and sponges. The interest in these two groups of animals stem from the following reasons:

a. The underwater observations that soft corals and many sponges appear to be remarkably free of predators, an observation which led to the hypothesis that fish toxins might be responsible for the protection of these animals.

b. The abundance of soft corals in the Gulf of Eilat (Red Sea) and the occurrence of many different species.

c. The fascinating spectrum of new molecular structures unveiled from sponges, and

d. The relatively high number of sponge species in the Gulf, many of which are unidentified as yet (the sponges belong to the phylum Porifera and comprise the most primitive multicellular invertebrate animals with an estimated 5000 described species).

## SOFT CORALS

The major faunistic and floristic components occupying space on the coral reefs of the northern Gulf of Eilat are stony corals, soft corals and benthic algae (Ref. 1). The average percent living coverage of soft corals (Octocorallia) on the reef flats of the northern Gulf ranges between 0.2 and 17%. Interestingly, seventy percent of the total living coverage of the soft coral community is contributed by 2-3 species belonging to the order Alcyonacea. They tend to form large single species "carpets" as those composed of Sarcophyton glaucum, Sinularia sp. and Lobophytum pauciflorum.

It was natural that many of the underwater observations in the area were directly connected with the soft corals such as the above mentioned ones, which gave rise to the hypothesis of repellents protecting soft corals against predators. Furthermore, following these observations, aquarium experiments demonstrated that not only were fish repelled by many soft corals but that they soon died if left in the tank with these animals. It was the toxicity towards fish, together with other biological activities (e.g. freedom from bacteria), which attracted our attention and served as a basis for monitoring the separation and purification of new compounds belonging to the cembranoid diterpenes (Ref. 2). The latter compounds are believed at least in part, to be responsible for the soft coral chemical defense mechanism (Ref. 3).

Quite a few, out of more than 150 well defined soft corals of the Gulf of Eilat, were explored by us. Working with soft corals requires, of course, a reliable convenient and preferentially rapid way for their identification. Classical taxonomy of soft corals involves careful examination of anatomical features (such as form, size and structure of spicules) and morphological features (such as growth forms). Quite often the identification is extremely difficult, and even opinions of different specialists may vary. Any additional cri-

teria that can aid in the systematic description of these soft corals, is therefore, of great advantage. A possible additional means suggested by us is based on the sesquiterpene (and other volatile compounds) composition of soft corals as determined by gas-liquid chromatography (Ref. 4). The "finger prints" thus obtained may serve as a useful and rapid complementary tool especially in cases where doubt exists due to variability in growth forms; although, of course, not as a substitute for classical taxonomy. Environmental conditions dictate to a large degree the growth forms of soft corals. In the Red Sea, our present state of knowledge indicates that the genus Sinularia consists of 25 species and the genus Sarcophyton consists of 12 species (Ref. 1). The GLC "finger prints" of these species may largely contribute to solving difficulties of identification of closely related species (e.g. see Fig. 1). Furthermore, this method may distinguish new species with greater ease than classical taxonomy. Indeed, the origin of the sesquiterpenes is intriguing as most of the soft corals live in symbiotic relationship with unicellular algae. However, this need not essentially diminish the value of the sesquiterpene "finger prints".

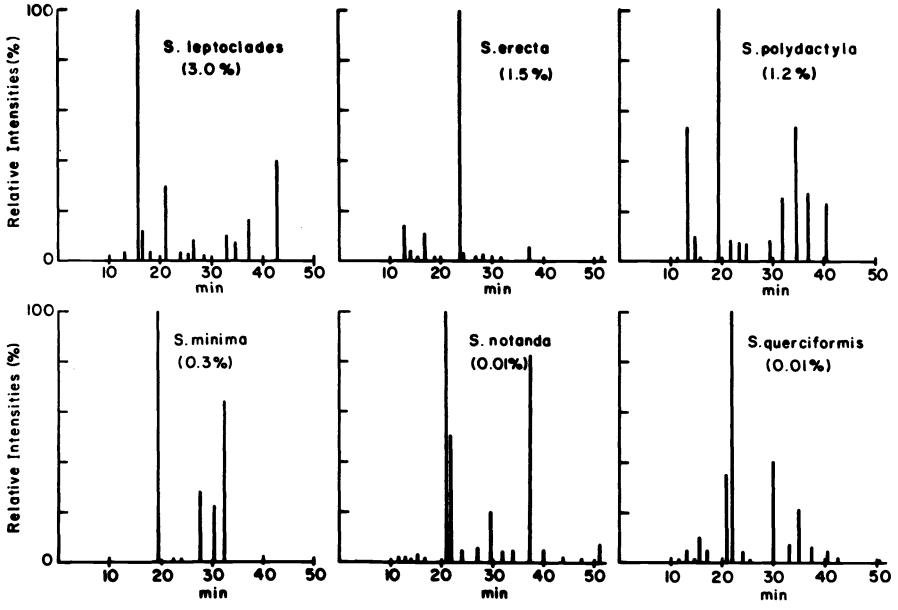
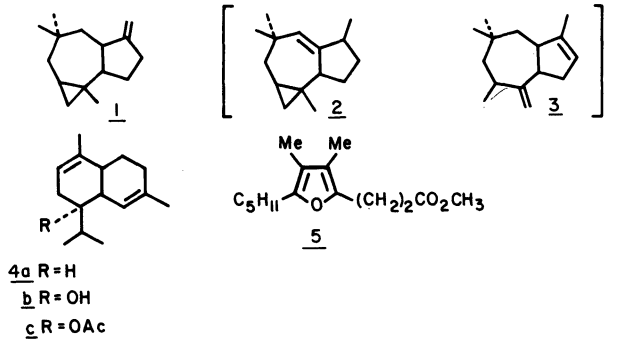



Fig. 1. Gas-liquid chromatograms of sesquiterpenes in six Sinularia species.

About a dozen of the sesquiterpenes isolated from different soft corals have been identified (Ref. 4). The new ones (1-4), together with methyl-3,4-dimethyl-5-n-pentylfurylpropionate (5) - another volatile component of the organic phase obtained during the freeze drying, follow (Ref. 5). Interestingly a whole series of furanoid long-chain fatty acids have been reported to be isolated from fish lipids (Ref. 6).




The main cembranoids which have been isolated by us from the genera Sarcophyton, Sinularia, Lobophytum and Alcyonium are shown in Fig. 2. If at the beginning of the research one obtained the impression that the cembranoids are widely spread within these animals, it was soon found that some of the soft coral species including the above mentioned genera, e.g. Sinularia and Lobophytum, do not contain diterpenoids in detectable amount (Ref. 7). Others, on the other hand (e.g. the genus Xenia) contain diterpenes but not cembranoids.

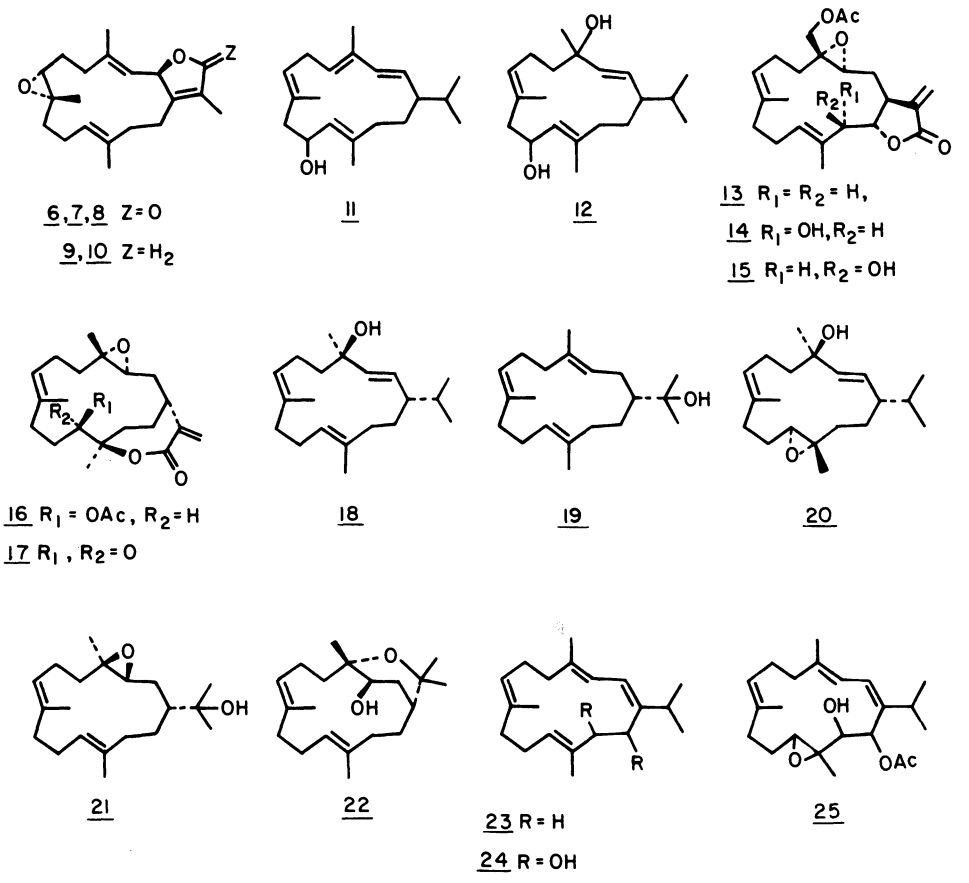
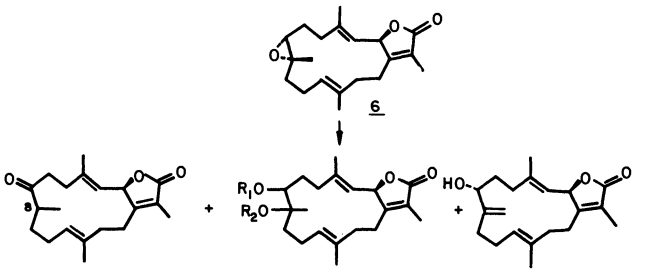


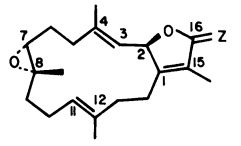

Fig. 2. Cembrane diterpenes from several soft corals

Sarcophyton glaucum was one of the first soft corals to be investigated, for the above mentioned reasons (Ref. 8). Specimens of this animal were found to contain up to 4% dry weight, of an epoxycembranolide, designated sarcophine (6). The complete structure of 6 was determined by X-ray diffraction and its conformation in solution by NOE measurements. The latter indicated that the preferred conformation of the macrocycle in solution does not differ much from the one observed in the solid state. Employing Uchida and Kuriyama's newly proposed chirality rule for the Cotton effect of butenolides (Ref. 9), we were able to assign the absolute configuration at position 2 of sarcophine (Ref. 10). Sarcophine selectively formed the 11,12-epoxide with peracid and the 11,12-dihydro derivative on hydrogenation indicating high stero and regioselectivity (selectivities which also were found with many other cembranes). Acid treatment of 6 resulted in a whole series of products depending on the acid used ($BF_3$ etherate; pTsOH, HOAc; pTsOH, MeOH; $H_2SO_4$, acetone; $HCO_2H$; $Al_2O_3$ in hexane and several Lewis acids) and also on the reaction conditions.



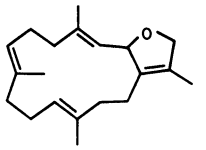
On several occasions we have isolated with sarcophine small amounts of two closely related compounds (7 and 8) which might be the 2-episarcophine and 2,8-bis-episarcophine (Ref. 11). An epimer of sarcophine was also recently reported by Coll from Sarcophyton crassocaule (Ref. 12). A way to elucidate the structures of these epimers and that of the closely related 16-deoxysarcophine (9) has been demonstrated by Faulkner (Ref. 13). Insufficient amounts of 7 and 8 have prevented us, so far, from their degradative structure elucidation. Whereas these episarcophines and two other alcohols, 11 and 12, appear in the soft coral in only minute amounts, 16-deoxysarcophine (9) appears in some specimens in considerable amounts (up to 4.5% dry weight). The structures of 9 and its epimer 10 were elucidated mainly by intensive decoupling experiments.

Variations in the chemical content of soft corals are well documented. Changes were observed not only in animals collected from remote locations (compare 34-36 with 6) (Ref. 14-16), but also from soft corals which grew up in the same habitat. Research aimed at shedding light on this phenomenon was undertaken by us and has been ongoing for the last three years, however, the results are far from satisfactory and can not be easily interpreted due particularly to experimental difficulties and to yet unknown reasons.

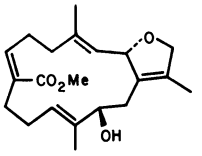


6 Z= O Sarcophine

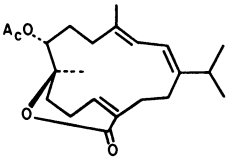
9 Z = $H_2$ 16-Deoxosarcophine



34 Sarcophytonin-A (Ref. 14)



35 Sarcoglaucol (Ref. 15)



36 Emblide (Ref. 16)

Different cembranoids from S. glaucum

Lobolide, another cembranolide (13) was isolated from Lobophytum crassum (Ref. 17). The nature of its individual functional groups was deduced from the $^{1}H$ and $^{13}C$ NMR spectra, which showed the presence of an α-methylenic γ-lactone, a trisubstituted epoxide, a primary acetate and two methyl-bearing trisubstituted trans double bonds. The relationships of the various functional groups were established mainly by decoupling experiments, isolation of levulinaldehyde from an ozonolysis experiment, as well as interpretation of lanthanide induced shift data. The structure of lobolide was recently confirmed by X-ray diffraction (see Fig. 3) (Ref. 18). Lobolide, like sarcophine, is toxic to fish.

L. Crassum is another example which illustrates the well documented variations in the chemical content of soft corals; from L. crassum collected at Leti Island, the Brussels group has isolated crassolide (Ref. 19) whereas isolobophytolide has been isolated by Coll and co-workers from L. crassum collected on the Great Barrier Reef (Ref. 20).

In addition to lobolide, two epimeric 13-hydroxylobolides, compounds 14 and 15 have been isolated by us most recently from the petroleum ether extract of L. crassum which was collected near Dahab, ca. 100 km north of the previous collection spot near Na'ama in the Gulf of Eilat (Ref. 21). Compounds 14 and 15 possess in addition to the functionalities of lobolide a secondary hydroxyl group. As with lobolide the relationship of the epoxy and lactone moieties was established by extensive decouplings of the proper protons. Furthermore, the latter experiment also determined the location of the additional hydroxyl in each one of the compounds, to be at C-13, thus compounds 14 and 15 are 13-epimers. The two compounds were found to behave differently under acidic conditions and on oxidation. Only one of the two (14) was oxidized by $MnO_2$. Jones oxidation on the other hand gave the same αβ-unsaturated ketone from the previously oxidizable epimer (14), while the other epimer (15) resulted in the 11,12-epoxy-13-keto derivative (Ref. 21). The different chemical behaviour of the two epimers can be best rationalized by the different stereochemistry around the 11,12-double bond (in relation to the rest of the macrocycle) as well as the 13-OH moiety. Whereas in epimer 14 the 13-H is readily removed to give the corresponding ketone, in the case of the other epimer (15) epoxidation of the double bond proceeds first, followed by oxidation of the alcohol. The above preference points to different reaction

profiles with each isomer and may thus suggest a restricted rotation of the reaction site and hence a quite rigid conformation of the entire macrocycle.

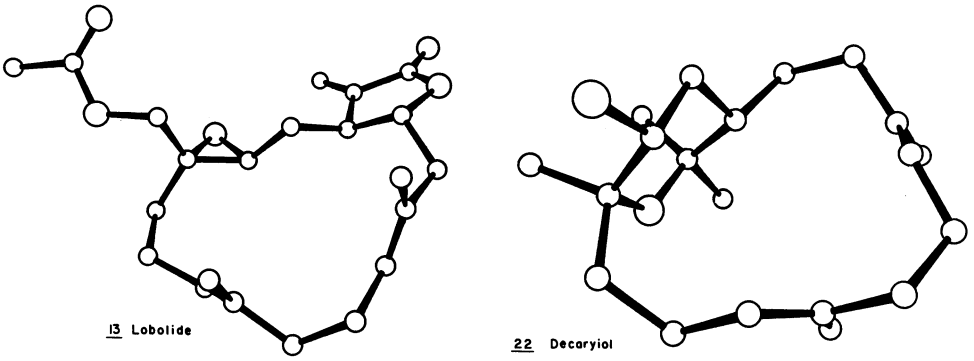
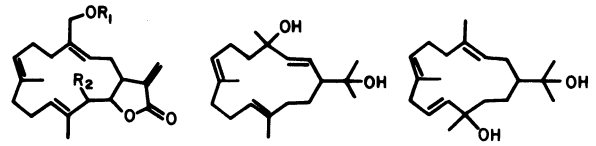



Fig. 3. ORTEP drawings of Lobolide (13) and Decaryiol (22)

Reinvestigation of the lipid extract of Lobophytum crassum revealed in addition to lobolide and the pair of the 13-hydroxylobolides, three new minor cembranoids (37, 38 and 39). The structures of the latter compounds were assigned on the basis of spectral evidence, comparison with lobolide, and extensive $^1H$ NMR decouplings. All three were found to be closely related to lobolide, namely the 3,4-deoxy, desacetyl- and 3,4-deoxy-lobolides (37 and 38 respectively), and desacetyl-13-hydroxylobolide (39).

From yet another Lobophytum species, L. pauciflorum, we have isolated in addition to nephthenol (19), two new compounds, designated paucifloro1-A and B (40 and 41 respectively). The structures of these two compounds were established by comparison of their NMR spectra and ozonolysis products with those of thunbergol (18) and nephthenol (19) and the new cembranoid alcyonol-B (45, vide infra).




37 $R_1=R_2=H$
38 $R_1=Ac, R_2=H$
39 3,4 epoxy-, $R_1=H, R_2=OH$

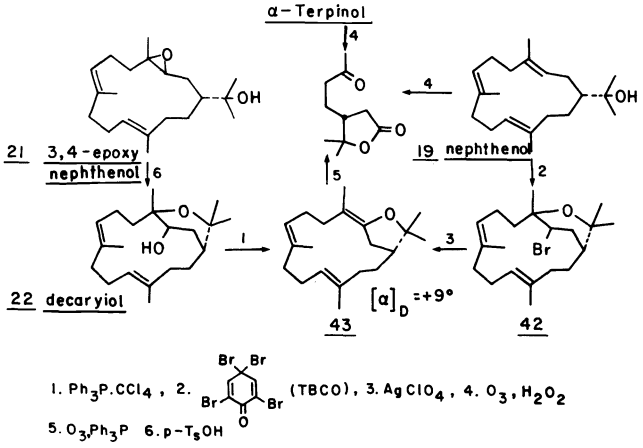
40 pauciflorol-A

41 pauciflorol-B

In the light of the very interesting cembranolides isolated from various Sinularia species (Ref. 22) and the high abundance of the latter animals in the Gulf of Eilat vide supra, it was of great interest to examine these organisms. Substantial amounts of diterpenes could be found in only one out of seven examined species, S. notanda. In S. querciformis we were able to identify trace amounts of 11-episinulariolide acetate (16) whereas all the others did not contain any diterpenes. The major diterpene in S. notanda was 11-episinulariolide acetate (16), which was found to be accompanied by 11-dehydrosinulariolide (17) (Ref. 7).

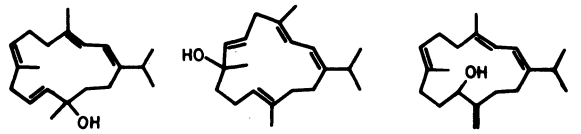
Repeated chromatography of the petroleum ether extract of yet another soft coral Sarcophyton decaryi yielded apart from large amounts of glycerides and steroids, five cembranoid diterpenes which were, in order of their polarity, thunbergol (18), nephthenol (19), trocheliophorol (20), 3,4-epoxynephthenol (21), and decaryiol (22) (Fig. 2). Among the five, three (20, 21 and 22) were not reported previously (Ref. 23 and 24). All the new compounds were fully characterized by spectral data, degradative studies by ozonolysis and other chemical transformations. Isolation of the 3,4-epoxynephthenol (21) and its acid catalyst transformation to decaryiol (22) (Ref. 24) strongly supports compound 21 as being a potential biogenetic precursor of 22. We have also examined several other epoxy hydroxy cembranes for their ability to undergo an acid catalyzed ether formation. Quite surprisingly the 13-hydroxy- lobolide pair (14 and 15) in contrast to (7E,11Z) -3,4-epoxy-13-hydroxy-7,11,15-cembratriene which was investigated by Faulkner (Ref. 25), failed to close to a tetrahydropyrane ring but rather produced a complex mixture. The rationale for this failure seemed to us to result from the rigidity of the macrocycle, which prevents the reaction sites from occupying the geometry required for the transannular reaction (this is in full agreement

with the above mentioned oxidation results of this pair). Of special interest are the transannular reactions which are believed to be responsible for the biosynthesis of oxygen bridged cembranes. The possibility of carrying out transannular reactions came to light in the early stages of the investigation of the marine cembranoids. Thus, bromination of eupalmerin acetate produced instead of the expected 3,4-epoxy-7,8-dibromide a 4,8-dibromo -3,7-ether (Ref. 26). The latter resulted from transannular participation of the epoxide oxygen in the reaction at the double bond (the bromo ether possessed the configuration expected for a concerted process). In 1976, Kato found tetrabromocyclohexadienone (TBCO) to be most efficient in the synthesis of mono bromo ethers starting from hydroxy cembrenes (TBCO serves as a source of the $Br^+$ ion and as the OH proton scavenger at the same time) (Ref. 27). The TBCO reaction simulates the natural hydroxy cembrane ether formation starting from hydroxy epoxides. This reaction was used by us in the synthesis of the 3-bromo analogue of decaryiol (42) starting from nephthenol (19) (interestingly, the same double bond is attacked by either the enzyme or by the laboratory electrophile ($Br^+$)). The latter reaction followed by elimination of HBr established the relationship between nephthenol and decaryiol determining thereby the absolute configuration at C-1 of decaryiol to be of the α-series. Recently the structure of decaryiol was confirmed by X-ray diffraction (see Fig. 3) (Ref. 28).




The relationship between compounds 18 and 20 was established by the Zn/Cu couple deoxygenation of the latter (20) producing thunbergol (18). As thunbergol, one of the terrestrial reported 2,7,11-cembratriene-4-ol isomers, is of known absolute configuration (Ref. 2), the absolute configuration of trocheliophorol was also established and found to belong to the α-series - the same configuration as was found for the other cembranoids of the S. decaryi.

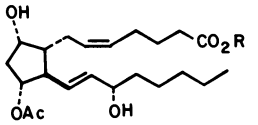
Among the examined soft corals from the genus Alcyonium (order Alcyonacea, family Alcyoniidae) was A. flaccidum (Ref. 21). From this animal, collected at Marsa-Hadamiya (Gulf of Suez, the Red Sea), we succeeded in the isolation of two known compounds, cembrene-C (23, 4.5% dry weight) (Ref. 29), and sarcophytol-B (24, 0.06% dry weight)(Ref. 14), together with 0.8% of a new cembranoid designated flaccidoxide (25). Like compounds 23 and 24, flaccidoxide has a UV maximum at 252 nm. The $^1H$ and $^{13}C$ NMR spectra point clearly to the common presence of the 1,4-tetrasubstituted diene (C-1 to C-4 and C-15 to C-18) in 23, 24 and 25. The NMR spectra also revealed that one of the two unconjugated double bonds present in compounds 23 and 24 was replaced in 25 by an epoxide. The distinction between the 7-8 and 11-12 double bonds could be made following a microozonolysis of 25, which afforded levulinaldehyde pointing to the 11,12-epoxy structure. At last, the two vicinal hydroxylated carbons were determined to be at C-13 and C-14 based on spectroscopic data. The structure of flaccidoxide was unequivocally confirmed following the formation of sarcophytol-B upon Zn/Cu deoxygenation of desacetyl flaccidoxide.



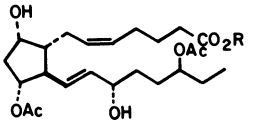

44 alcyonol-A 45 alcyonol-B 46 alcyonol-C

From Alcyonium utinomii we succeeded in the isolation of three new cembranoids designated alcyonol-A, B and C (44, 45 and 46 respectively). All three were found to be closely re-

lated to cembrene-C. The presence of the 1,1,4,4-tetrasubstituted conjugated diene in 44, 45 and 46 was implied by the presence of $^1H$ NMR signals around δ6.0 (two doublets with a coupling constant of 11Hz), IR(1660-1670 $cm^{-1}$) and UV (248-252 nm) absorptions. The conformation of the diene was deduced as s-trans on the basis of the similarity of the data between the above three, cembrene-C, sarcophytol-D or E (Ref. 30). The NMR spectra suggested also two other functionalities, $-CH_2CH=CHC(OH)(CH_3)$ and $-CH=C(CH_3)-$. The above data together with only one double allylic methylene in molecules 44 and 45 agree with two proposed structures only. The differentiation between the 1,3,7,10-tetraene-12-ol and the 1,3,6,11-tetraene-8-ol was achieved following a microozonolysis experiment (comparison with levulinaldehyde and 2-methyl-hepta-3,6-dione as well as $^1H$ NMR decoupling experiments). The $^1H$ NMR spectrum of the third compound (46) indicated in addition to the diene, the presence of a trisubstituted double bond ($-CH=C(CH_3)-$) and an allylic alcohol $-CH_2CH(OH)C=CH_2$. Ozonolysis of compound 46 gave inter alia levulinaldehyde, and Jones oxidation - the corresponding αβ-unsaturated ketone. Based on the above results and the assumption of biogenetic similarity between 46 and other cembranoids of this soft coral, the structure of this alcohol was proposed to be the (1, 3E, 7E, 12(20))-11-hydroxycembratetraene. We prefer the 11-ol over the 13-ol, which cannot be excluded, on basis of the good agreement between the $^{13}C$ NMR spectra of 46 and the relevant parts of sarcophytol-E and cembrene-C.

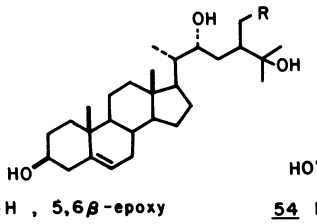



47 R=Me
48 R=H

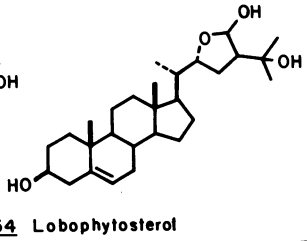



49 R=Me
50 R=H

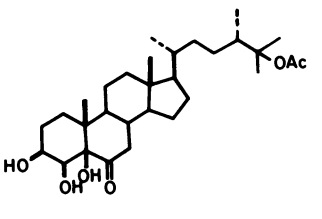
Beside the cembrane diterpenes (Fig. 2) several other interesting groups of compounds have been discovered. From Lobophytum depressum we have isolated 15S-$PGF_{2\alpha}$-acetate methyl ester (47) together with its oxygenated 18-acetoxy derivative (49) (Ref. 31). The structure elucidation of the new 18-hydroxylated $PGF_{2\alpha}$ (49) was achieved basically from its $^1H$ and $^{13}C$ NMR spectra. The $^1H$ NMR spectrum clearly indicated an additional OAc-group in 49 in comparison to 47. Furthermore the less intense $CH_2$ absorption of the $C_5H_{11}$ segment, together with the more explicit triplet shape of the terminal C-20-Me group, suggested that the new OAc group was located on the "lower" chain. Unequivocal proof for the 18-OAc location was obtained from the $^{13}C$ NMR spectrum; comparison of the chemical shifts of $C_{16}$-$C_{20}$ in 47 and 49 which clearly indicated C-18 to be the hydroxylated site (proper β and γ-effects on the neighboring carbons). Beside the methyl esters, 47 and 49, the soft coral also contained small amounts of the corresponding free acids (48 and 50 respectively). Our discovery of prostaglandins in a soft coral came almost at the same time as their being found in the alga Gracilaria lichenoides, another marine organism and the first plant source of PGs (Ref. 32). Both disclosures came almost a decade after PGs were first reported from a marine organism, namely, their isolation from the gorgonian Plexaura homomalla (Ref. 33) and after many fruitless efforts to find more PGs by different groups.




51 R=H , 5,6β-epoxy
52 R=OH Depresosterol
53 R=OH , 5,6β-epoxy




54 Lobophytosterol
55 5,6β-epoxy

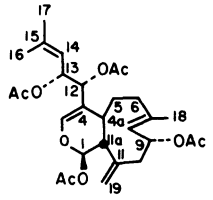



56 Lobosterol

From L. depressum we succeeded also in the isolation of five new 28-oxygenated $C_{28}$-sterols which are of particular interest from the biogenetic point of view (51-55) (Ref. 34). 24-Methylcholesterols and 24-methylenecholesterols are by far the most abundant sterols in Alcyonaceans (Ref. 35). Many of these sterols possess the 5α, 6β-dihydroxy grouping while others contain in addition the 25-hydroxyl (e.g. lobosterol (56) isolated from Lobophytum pauciflorum (Ref. 36)). The above $C_{28}$ sterols are believed to be produced in the animal tissue, by methylation of the cholesterol side-chain. Some animals including probably some coelenterates, too, have the ability to dealkylate $C_{28}$ sterols to produce cholesterol

(Ref. 37). In this connection, it was of particular interest to discover new sterols possessing oxygenated C-28 groupings which may be intermediates in the possible demethylation pathway. Five new sterols all belonging to the polyoxygenated $C_{28}$ category in which the C-28 atom appears as a $CH_3$, $CH_2OH$ or CHO group have been isolated from L. depressum. The structures of these novel compounds, 5β,6β -epoxy-24ε -methylcholestan-3β, 22(R),25-triol (51), depresosterol (52), lobophytosterol (54) as well as the 5β,6β-epoxides (53 and 55) of the latter two compounds, were determined mainly on the basis of the fully interpreted $^1H$ and $^{13}C$ NMR spectra as well as the mass spectra and several chemical transformations which resulted in unambiguous structures. The above sterols are the first examples of marine 22,28-oxygenated sterols.

Until recently the only large group of diterpenes isolated from soft corals were the cembranoids (Ref. 38). In 1977, Schmitz reported the isolation, from Xenia elongata, of a new compound, xenicin (57), with a novel carbocyclic skeleton (Ref. 39). Since then many other compounds with the same carbocyclic skeleton as well as other closely related ones, were isolated from Xenia species (Ref. 40). We believe this group will soon become another prominent class of marine diterpenoids. Included in this group are the xenicins, xeniolides



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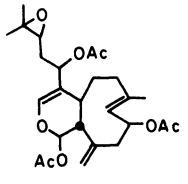

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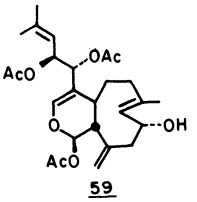

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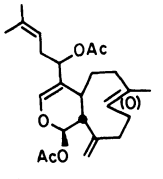

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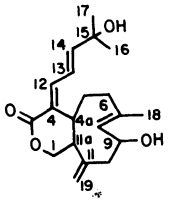
61 7,8-epoxide



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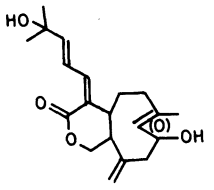

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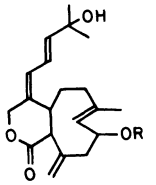
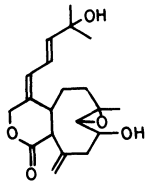
64 7,8-epoxide



65 R = H

66 R = Ac



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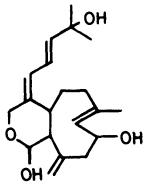

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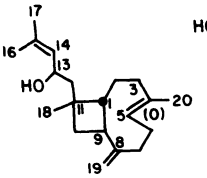

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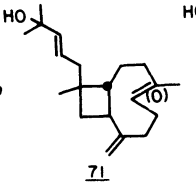
70 4,5-epoxide



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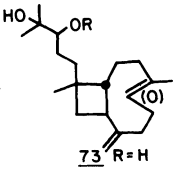
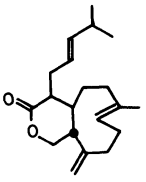
72 4,5-epoxide



73 R = H

74 R = Ac

75 R = Ac 4,5-epoxide



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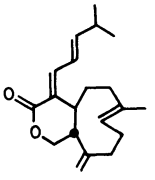

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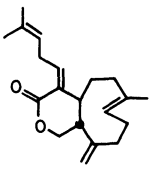

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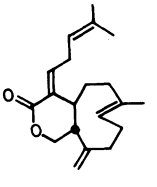

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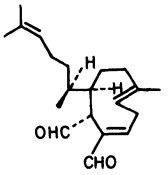

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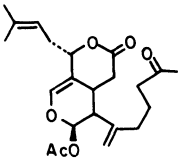


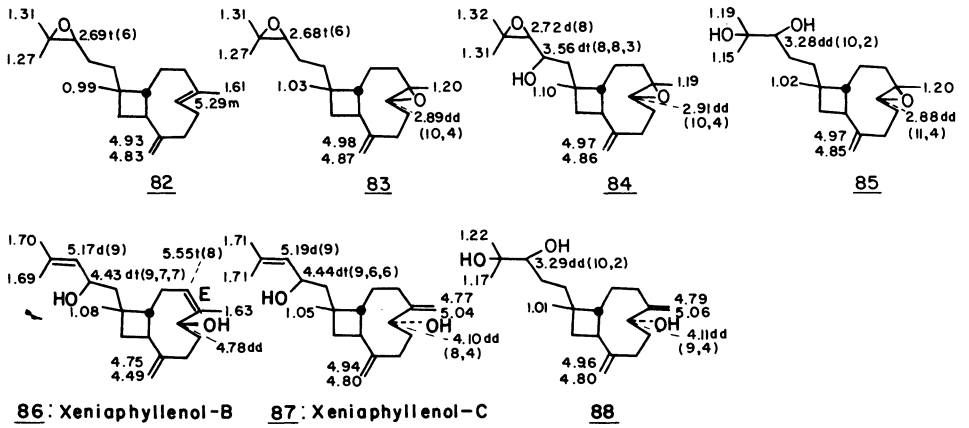
81

and the biogenetically closely related xeniaphyllanes. About twenty members of the group have already been found in four different Xenia species (57-75) and several others, isolated from Xenia macrospiculata, X. obscuronata and X. lillelae will be reported here for the first time. The isolation of the above mentioned compounds from the latter three Xenia species as well as from X. elongata (Ref. 39) and X. novae-britanniae (Ref. 41), seems to indicate that the presence of xenicin-related diterpenoids (including the xeniaphyllanes) is a regular and distinctive chemotaxonomic feature of the genus Xenia, at least to the same extent as to which the cembranoids are a chemotaxonomic feature of the genera Sarcophyton, Sinularia and Lobophytum. This does not mean, of course, that all the Xenia species will contain the xenicins and that, on the other hand, those compounds will not be found in other organisms. It was thus with great interest that diterpenes with the xeniolide skeleton, the coraxeniolides (76-79) were reported by Scheuer and co-workers from the Hawaiin pink coral, Corallium sp. (Ref. 42). Furthermore, it was pointed out by the authors that the gorgonian from which these metabolites have been isolated is free from symbiotic photosynthetic algae. The coraxeniolides therefore must be biosynthetic products of the animal or ingested with its diet. Whether this is also the case with the Xenia species which live in symbiosis with microorganisms is unknown. Coll and co-workers reported most recently the isolation of xeniaphyllanes from a Nephthea species (Ref. 43). A closely related substituted cyclononane diterpene, dictyodial (80), was found by Finer et. al. in the brown algae Dictyota crenulata and in D. flabellata (Ref. 44). Yet another interesting compound which was most recently isolated from a soft coral, Alcyonium sp. from Okinawa, is alcyonolide (81) (Ref. 45). The carbon skeleton of the latter compound (81) corresponds to a seco-type variety of xenicin. The biogenetic pathway of alcyonolide (81) is presumed to proceed after completion of a xenicin-type carbon skeleton.

The various Xenia metabolites can be divided into two major parts; the first part deals with the 2-oxabicyclo[7.4.0] tridecanes, the xenicins and the corresponding lactones, the xeniolides (57-68), while the second part describes the xeniaphyllanes possessing the bicyclo[7.2.0] undecane structure (69-75). The classification of the various compounds, in one of the above structural groups, is achieved quite easily according to the $^{1}H$ and $^{13}C$ NMR data. It can be seen that the xenicins, for example, exhibit in the $^{1}H$ NMR only three Me groups, in comparison to four in the spectra of the xeniaphyllanes, and show in the low field region two very characteristic sharp doublets belonging to H-1 and H-3. The $\delta_C$-values of the caryophyllene bicyclo[7.2.0]-undecane skeleton in compounds 69-75 are, on the other hand, very helpful in the xeniaphyllanes structure elucidation. Characteristics also include MS-fragmentations of the bicyclic skeleton as well as the cleavage patterns of the side chains.

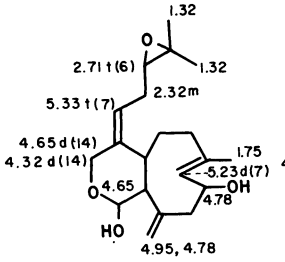
The biosynthesis of the xenicins is suggested to start either from cyclization of geranylgeraniol pryophosphate or geranyllinalool pyrophosphate, or alternatively, from the oxidative cleavage of the four-membered ring of a proper xeniaphyllane (Ref. 39). Xenialactol-C (68), or another compound with the same heterocycle, is suggested to be an intermediate between the xenicins and the xeniolides.

Several new xeniaphyllanes, which were isolated from new batches of Xenia lilielae (82-85), X. macrospiculata (85-87) and X. obscuronata (85 and 88), are reported here for the first time. Xeniaphyllenol-oxide (70) could be transformed chemically, in a way similar to the method developed by Dev (Ref. 46), to xeniaphyllenol-C (87). The structures of the rest of the molecules were assigned on the basis of their proton and $^{13}C$ NMR spectra, assisted, to a large extent, by comparisons with the corresponding caryophyllane derivatives (Ref. 47). As can be seen from the formula of compounds 69-75 and 82-88, the main variations in their structures are in the side chain and in the close proximity of the 4,5-double bond.

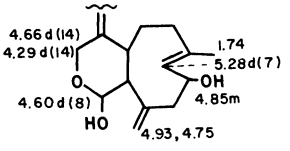


All three Xenia species which were examined by us were found to contain varying amounts of another new diterpene designated xenialactol-D (89). The bicyclic skeleton of the latter was determined by comparison of its NMR data with those of xenialactol-C (68). The structure of the side chain was clear from the chemical shifts of the terminal Me-groups as well as from the $\delta_C$ values of C-14 to C-17 and decoupling of H-12, 13, 13' and 14.

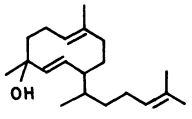
In addition to the above diterpenes, we have also isolated two sesquiterpenes and two diterpenes of another class. Obscuronatin (90), one of the diterpenes, was previously reported by us and it was found to rearrange to compound 91 by $Ph_3P{\cdot}CCl_4$. The latter compound (91) (Ref. 48) has now also been identified (by $^1H$ NMR) in the crude Xenia sesquiterpene fraction together with palustrol and 7-acetoxymuurolene (4c). These two compounds were previously isolated by us also from several other soft corals (Ref. 4).



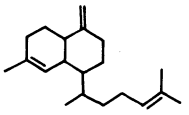

89: Xenialactol - D




68: Xenialactol - C




90: Obscuronatin



91

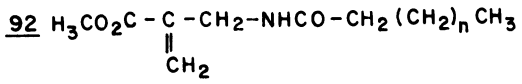
## SPONGES

Sponges have been proven to be particularly rich in bioactive compounds (Ref. 49). We focused our screening of sponges, of the Gulf of Eilat, mainly on sponges with antimicrobial activity and ones containing interesting secondary metabolites. Our studies of several of the more interesting new metabolites, isolated from sponges during the last years, follows. Included among the compounds are cyclic peroxides, polyacetylenes, new scalarins, alkylated scalarins, novel fish toxins, the latrunculins A and B, several yet unreported sipholanes and the new dibromotyrosine metabolites psammaplysin-A and B.

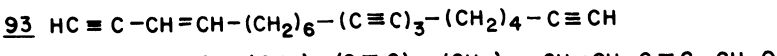
Among the explored sponges was the black sponge Fasciospongia cavernosa, collected in the northern part of the Gulf of Eilat, which was found to contain in remarkably high concentration (1-2% dry weight) a new, naturally occurring β-amino acid (92) (Ref. 50). This acid does not appear, however, in the free form but rather in a series of N-acyl methyl esters. Acid methanolysis of the natural product afforded the acids' methyl esters, 5 of which were separated and identified by the GC-MS technique. The 2-methylene-β-alanine methyl ester was fully characterized by its $^1H$ NMR spectrum as well as by well defined MS-fragmentation patterns. Recently, several other N-acyl derivatives of this amino acid were isolated by Scheuer from an unidentified sponge and their oxygeneted long chain acids had been identified (Ref. 51).

Attracted by the characteristic polyacetylenic IR and UV absorptions of the crude petroleum ether extract of the sponge Siphonochalina sp., we undertook the elaborate chromatographic separation and structure elucidation of this extract (Ref. 52). Whereas polyacetylenes were well known constituents of plants when this research started (Ref. 53), almost none have been disclosed from marine organisms. We have succeeded in the isolation and the structure elucidation of six new compounds from the complex mixture. Other minor components did not withstand the purification process - not surprisingly for polyacetylenes. All characterized compounds were n-$C_{22}$ straight chain acetylenic compounds possessing, except for one ($\Delta^{15}$-docos-1-yne) a cis enyne terminus on one side of the chain and either a terminal acetylene or a propargyl alcohol on the other end. Two of the identified materials are compounds 93 and 94 (see Fig. 4). While the termini of both 93 and 94 were determined by their $^1H$ NMR spectra, the existence of a triyne was unequivocally confirmed in each one of the two by the highly characteristic UV spectra with its sharp vibrational fine structure. Assignment of the location of the triynes, however, required further experimental data. A LIS experiment determined the exact site of the latter moiety in each

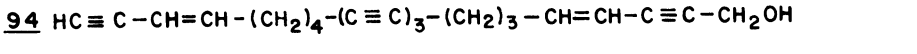
compound. While a complexation site was already embodied in alcohol 94, an apoxide, prepared by epoxidation of the double bond of 93, served in 93 for the same purpose. Of interest is the enyne terminus which appears in many marine metabolites and is not unknown in terrestrial sources.



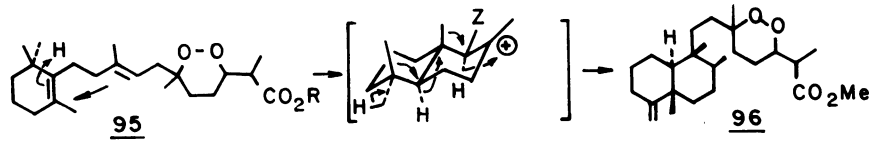
**92** $H_3CO_2C-C(=CH_2)-CH_2-NHCO-CH_2(CH_2)_nCH_3$

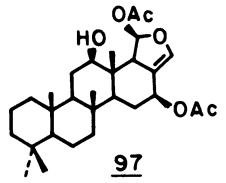


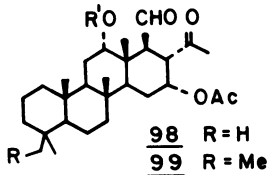
**93** $HC\equiv C-CH=CH-(CH_2)_6-(C\equiv C)_3-(CH_2)_4-C\equiv CH$

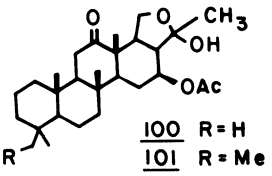


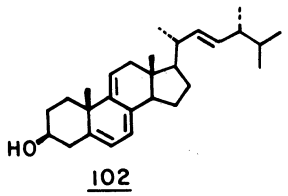
**94** $HC\equiv C-CH=CH-(CH_2)_4-(C\equiv C)_3-(CH_2)_3-CH=CH-C\equiv C-CH_2OH$



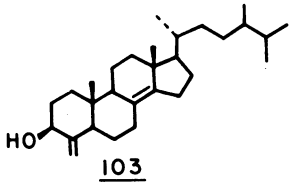
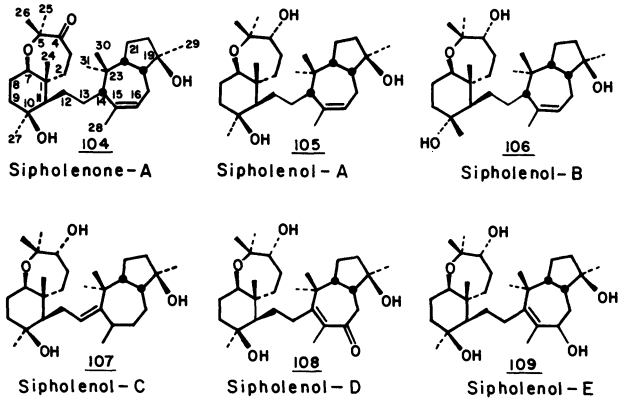




Fig. 4. Several metabolites from sponges

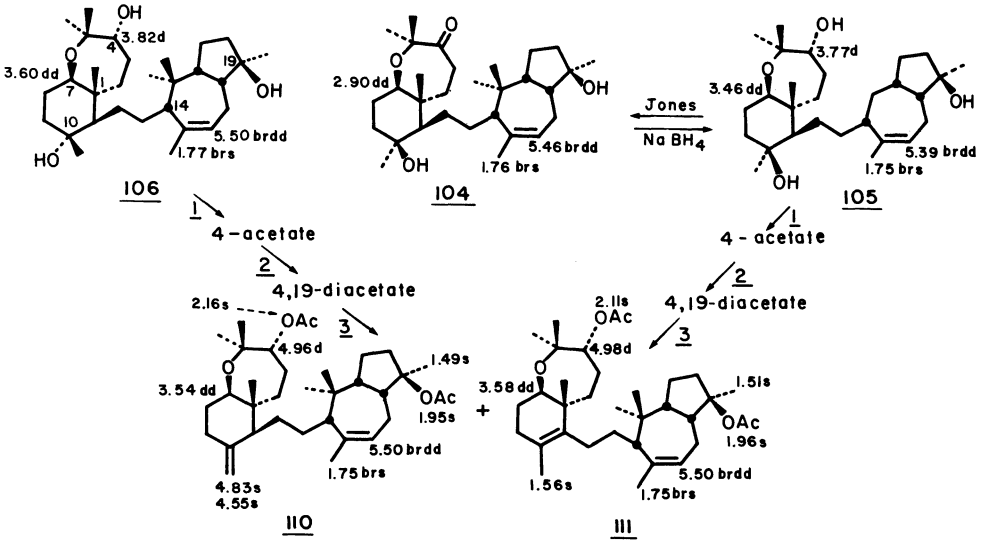
The number of natural cyclic peroxides increased remarkably during the last few years mainly due to the discovery of quite a few from marine organisms. Two peroxides isolated from Prianos sp. from the Gulf of Eilat, are the $C_{24}$-isoprenoids 95 and 96 (see Fig. 4) (Ref. 54). The structure of compound 95, designated muqubilin (after the place of collection, Marsa el Muqebla) was determined following full spectroscopic ($^1H$ NMR and mass spectra) characterization of 95 and two of its ozonolysis degradation products. The structure of the second compound, 96, was proposed following a report by the Belgian group of the structure of sigmosceptrellin-A, isolated from Sigmosceptrella laevis (Ref. 55). Comparison of the reported spectroscopic data for the latter with those of compound 96 suggested it to be a stereoisomer. A possible biosynthesis of this tricyclic metabolite starting from muqubilin (95), is suggested.

The scalarins are tetracarbocyclic sesterterpenes which were isolated from several sponges (Ref. 49). Heteronemin (97) for example, is one of this groups' members which was isolated from Heteronema erecta collected both in Australia (Ref. 56) and in the Gulf of Eilat (Ref. 57). Several other closely related structures (98 - 101) were isolated recently from the sponge Dysidea herbacea collected in the Gulf of Suez (Red Sea) (Ref. 58). The extraction of the freeze-dried sponge and subsequent chromatography gave 3 pairs of compounds: scalarherbacin-A and B (98 and 99), the corresponding acetates of the latter two and scalardysin-A and B (100 and 101) (see Fig. 4). The mass and $^1H$ NMR spectra determined unequivocally that each pair consists of two homologues, possessing the same functionalities and differing only in the substituents at C-4. Partial separation (up to ca. 80% enrichment of each one of the compounds) of the two homologues was achieved on a RP-18 reverse phase TLC plate or HPLC column. The structures of the pairs were determined from their spectral data mainly on the basis of their mass, $^1H$ and $^{13}C$ NMR spectra. Similar $C_{26}/C_{27}$ scalarins were also reported recently by Kitagawa from the sponge Phyllospongia foliascenes (Ref. 59) and by Kazlauskas and co-workers from two other Phyllospongia spp. (Ref. 60).

Many of the examined sponges were found to contain complex mixtures of sterols, a few of which were analyzed by Djerassi's group in Stanford. The steroidal components were fractionated through RP-HPLC and analysed by a combination of physical methods, including high resolution GC/MS and 360 MHz $^1$H NMR. Two of the new sterols ergosta-5,7,9(11), 22-tetraen -3β-ol isolated from the sponge Biemna fortis (Ref. 61) and conicasterol, 4-methylene-24(R) -methylcholest-8(14)-en-3β-ol, isolated from the sponge Theonella conica (Ref. 62) are shown in Fig. 4 (102 and 103, respectively).



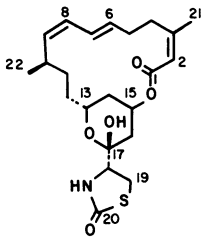

Terpens are the most abundant nonsteroidal secondary metabolites isolated from marine sponges (Ref. 49). Although a $C_{30}$ compound, mokupalide, a hexaprenoid, was isolated from a sponge (Ref. 63), to the best of our knowledge, the sipholanes, vide infra, are the first triterpenes, aside from squalene, to be isolated from a sponge. Until now six compounds possessing the new sipholane carbocyclic skeleton were isolated from the sponge Siphonochalina siphonella. The structure of one of the compounds, sipholenol-A acetate, obtained from the natural alcohol 105 by $Ac_2O$/Pyridine acetylation, was determined by X-ray diffraction analysis (Ref. 64). A structure for four other sipholane triterpenes designated sipholenone-A, sipholenol-B,C and D (104, 106, 107 and 108, respectively), is proposed. Compounds 104 and 106 were intercorrelated by chemical transformations. The suggested structures for 107 and 108 are based on their spectral data while the structure of the sixth compound 109 is tentative.



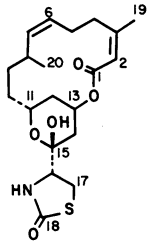

1. $Ac_2O$/Pyr 12 hrs 2. $Ac_2O$/Pyr 5 days 3. $SOCl_2$ / Pyr 0°C

Among the most prominent sponges in the Gulf of Eilat are colonies of the branching red-coloured Latrunculia magnifica Keller, occurring at a depth of 6.0 to 30.0m, and clearly observable under water from relatively long distances. As reported by us previously, colonies of this sponge were never observed to be damaged or eaten by fishes. Furthermore, when squeezed manually, these sponges exude a reddish fluid, a "juice" which causes fish to escape immediately from the sponge vicinity. When L. magnifica is squeezed

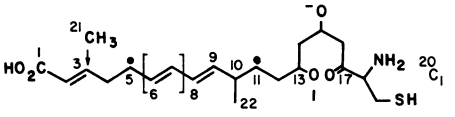
into an aquarium it causes poisoning and death of the fish within 4-6 minutes (Ref. 65). Two new toxins, the latrunculins (112 and 113), were isolated from this sponge. A full structure determination of these toxins, by spectroscopic methods, was aided by an X-ray diffraction analysis of a crystalline derivative of latrunculin-A (112). The Latrunculins exhibit a new class of 14 and 16 membered macrolides to which the rare 2-thiazolidinone moiety is attached. A suggested biogenesis of the latrunculins is shown in formula a. The toxins are proposed to be polyketides possessing the cysteine molecule as the starting acyl group (Ref. 66).



112 Latrunculin-A

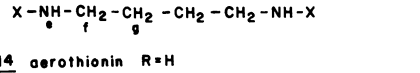


113 Latrunculin-B

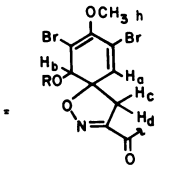


a

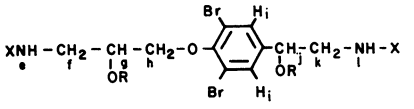
Latrunculin-A and B have been evaluated with regard to their effects on cultured mouse neuroblastoma and fibroblast cells. The mouse neuroblastoma clone N1E-115 cells can express many neuronal properties and are amenable to detailed morphological, electrophysiological and biochemical studies. They provide, therefore, a useful system for exploring the action of toxins on a variety of cellular functions. Spector and Shochet (Ref. 67), have found that submicromolar concentrations of latrunculins cause morphological changes in neuroblastoma cells. Immunoflourescence studies using antibodies specific against actin and tubulin reveal that the latrunculins induce profound changes in the organization of microfilaments in the cells while leaving the organization of microtubules unaltered. These toxins exert a similar action on fibroblasts although at somewhat higher concentrations. Upon toxin removal, both types of cells rapidly recover their normal morphology and microfilament organization. The molecular mode of action of these unusual marine natural products is still unknown, but their effect strikingly resembles those of the mold metabolites cytochalasins, the only class of drugs known to specifically disrupt microfilamentous structures. The latrunculins, however, possess another mode of action and act at least ten fold lower concentrations.



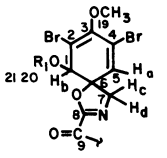
114 aerothionin R=H



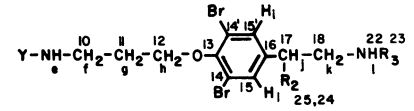
X=



115 fistularin-3 R=H



Y=



116 $R_1=R_2=R_3=H$ Psammaplysin-A

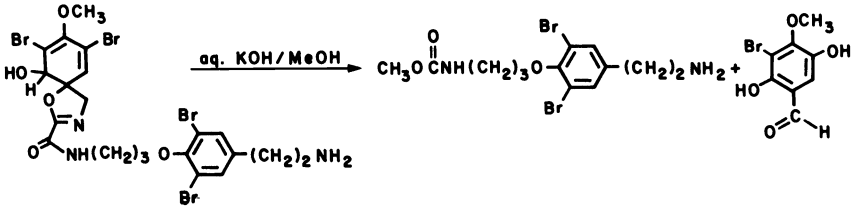
117 $R_1=R_3=H, R_2=OH$ Psammaplysin-B

118 $R_1=R_3=COCH_3, R_2=H$

121 $R_1=R_2=H, R_3=COCH_3$

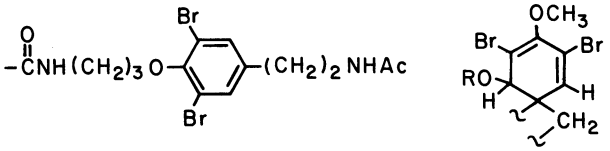
Brominated metabolites from sponges of the genus Aplysina (=Verongia) family Verongiidae include active antibacterial phenols and quinones which are proposed to be derived from mono or dibromo tyrosines. Two representatives of these unusual brominated compounds are aerothionin (114) (Ref. 68) and fistularin-3 (115) (Ref. 69). Studies of the metabolites of Psammaplysilla purpurea, another sponge from the Verongiidae family which was collected by us in the southern part of the Gulf of Eilat, resulted in the isolation of two new antibacterial compounds which were designated psammaplysin-A (116) and -B (117) (Ref. 70). Both compounds (116 and 117) exhibited in vitro activity against gram positive as well as E. coli bacteria. Psammaplysin-A was obtained as a foam following chromatography, on a Sephadex LH-20 column, of the MeOH extract (the Sephadex column was prepared and eluted with a $CHCl_3$; MeOH 1:1 mixture). Psammaplysin-A (116) gave a crystalline diacetate (118) with satisfactory elemental analysis for $C_{25}H_{27}Br_4N_3O_7$.

The $^1H$ NMR spectrum of the diacetate (118) suggested the presence of $ArCH_2CH_2NHCOCH_3$ (2.75t, J=7 Hz, 3.45dt, J=7 Hz, collapsing to a triplet on $D_2O$ exchange of the amide proton, 5.80bt, J=7.5 Hz and 1.95s, 3H) and $-CONHCH_2CH_2CH_2OAr$(7.16 bt, J=6Hz, 3.70dt, J=6 Hz, collapsing to a triplet on $D_2O$ exchange of the NH, 2.10 quin, J=6 Hz and 4.08t, J=6 Hz). The aromatic ring in both units is proposed to be the same dibromophenoxy group which appears in fistularin-3 (115). The latter ring is thus also responsible for the two proton singlet at δ7.35, which remains almost unchanged in various degradation products, and is in full agreement with the $^{13}C$ NMR spectrum. The presence of the following functionalities in the rest of the molecule of 118; CHOAc (6.40s and 2.22s), an isolated $CH_2$ group (as AB quartet at 3.02 and 3.23, J=15.9 Hz), a methoxy (3.67s) and a single vinyl proton (7.05s) was suggested by the NMR spectrum. These data together with the expected elemental composition of this part of the molecule (according to the elemental analysis of 118 and the structure of a) suggested that unit b which is part of compounds 114 and 115 is also part of compound 116. Comparison of the $^1H$ NMR spectrum of compounds 114, 115 and 116 pointed to a remarkable similarity, but not identity, of the $^1H$ resonance lines belonging to moiety b in the various compounds. The same was true also with the $^{13}C$ NMR data.




116 119 120

Mild basic treatment of compound 116 (or 117) brought about aromatization of the cyclohexadiene moiety (disappearance of the CHOR singlet). Two compounds 119 and 120 could be isolated from the reaction mixture. This reaction also clearly indicated the absence of the isoxazole ring. If the isoxazole ring was part of compound 116 as is the case with compounds 114 and 115, the basic conditions would be expected to open up this ring to give a hydroxyl amine (Ref. 68) which could not be detected in the case of 116. Furthermore, mild $LiAlH_4$ treatment, known to reduce isoxazoles, did not affect compound 118 except for hydrolising the O-acetyl group to give the alcohol 121. The above data point to the following two moieties:

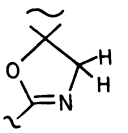


a b

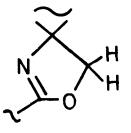
These two structures account for all of the molecule's atoms except for one carbon, a nitrogen and one oxygen atom. Three possible structures could be suggested for the missing heterocycle which has to replace the isoxazole ring of compounds 114 and 115, in order to link the above two units to give molecule 116. Of the three, we preferred structure c on the basis of the $^1H$ and $^{13}C$ NMR chemical shifts of the $CH_2$ moiety.

The suggested structure is in full agreement with the $^{13}C$ NMR chemical shifts, assigned on the basis of the δ-values, SFORD study and comparison with the degradation products and aerothionin (114). Psammaplysin-B (117) differs from psammaplysin-A by possessing an

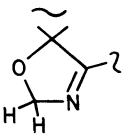
additional OH-group next to the dibromophenoxy ring - as was clear from its NMR spectra.



c



d



e

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