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# RECENT DEVELOPMENTS IN RESEARCH ON METABOLITES FROM CARIBBEAN MARINE INVERTEBRATES

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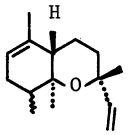
## INTRODUCTION

As a consequence of earlier surveys[1] of extracts of a broad spectrum of marine invertebrates for pharmacological activities, our natural products research has been focused over the years on the chemistry of sponges, gorgonians, and sea hares. Sponges and gorgonians offer the practical advantage that they are frequently abundant and hence trace metabolites can be pursued where high levels of activity indicate that the effort of large scale work is worthwhile. Sea hares offer the advantage that they are often rich sources of interesting metabolites, but at the same time they pose a problem of determining the identity of the ultimate source, usually algae[2], of these new natural products. The problem is compounded by the fact that the sea hare's algal diet presumably varies seasonally, and hence complicated mixtures accumulate. Nevertheless, as sources of diverse natural products, sea hare digestive glands are difficult to match.

In this paper we would like to summarize some of our recent work relating primarily to sea hares and sponges. In most cases specific studies were initiated because some pharmacological activity had been detected in a crude extract. In one instance, only the high levels of cytotoxicity revealed by bioassay during fractionation kept us working on what appeared to be a decidedly uninteresting mixture as judged by the natural product chemist's usual criteria, NMR analysis. Ultimately, this search led us to a very active trace component, whose structure is the most unusual of those described in this paper.

## SEA HARES

Our attention was first drawn to the sea hare *Aplysia dactylomela* by a report[3] that extracts of this animal showed both cytotoxicity and *in vivo* antitumor activity. Our first collection of *Aplysia dactylomela*, which reaches sizes up to 60 cm in length, was made at Bimini in the Bahamas. The extracts proved to be cytotoxic and showed mild *in vivo* tumor inhibitory activity. Bioassay-guided fractionation has resulted in the isolation of a variety of biogenetically unrelated compounds and these will be reviewed here briefly to put into perspective more recent work. The first isolates were a group of sesquiterpene ethers having a pleasant odor which we designated dactyloxene-A, -B, and -C. On the basis of spectral analysis and some chemical modifications, we proposed the structures 1, 2, and 3, respectively, for these compounds[4], in which the relative stereochemistry was unresolved at the starred centers in 2 and 3. Recently, Maurer *et al*[5] have confirmed the structure assignments for 2 and 3 by total synthesis and have established the relative stereochemistry shown at the starred centers. Isolated along with the sesquiterpene ethers 1-3, was the related alcohol, dactylenol (4), and its acetate (5). In addition to these, a new bicyclic alcohol, dactylol (6), was obtained[6] which possesses a unique rearranged sesquiterpene carbon skeleton.

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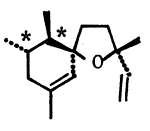


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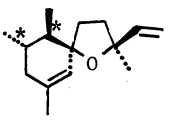


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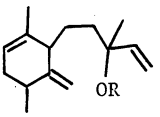
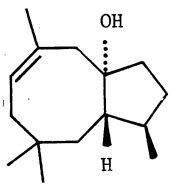



4 R = H

5 R = Ac

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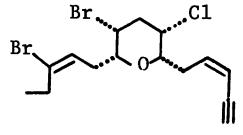
A variety of halogenated metabolites have also been isolated from *A. dactylomela* collected at Bimini. The first of these were dactylyne (7)[7] and isodactylyne (8).[8] Dactylyne (7) was found[9] to be a potent inhibitor of pentobarbital metabolism and may be a more general drug metabolism inhibitor. Isodactylyne (8) has virtually the same activity as dactylyne, while the hydrogenation product of dactylyne, octahydromonodebromodactylyne (9), shows no significant effect on pentobarbital metabolism.[10]

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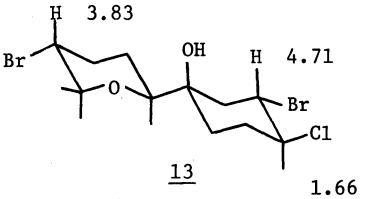


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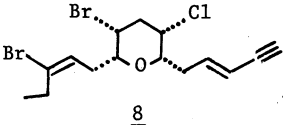


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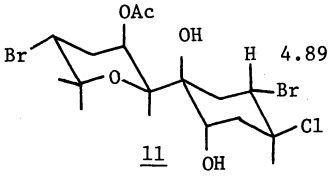


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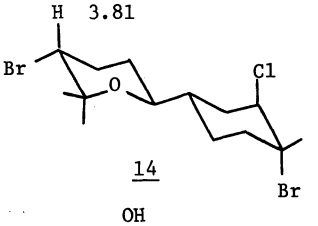


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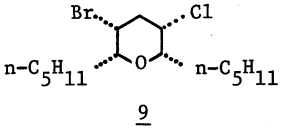


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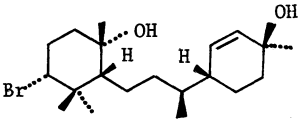


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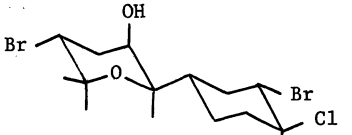



15

1.67

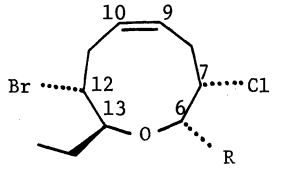
Trace amounts of several halogenated sesqui- and diterpenes were also isolated. The structure of the sesquiterpene deodactol (10) was secured by x-ray diffraction[11], while that of the companion compound, dihydroxydeodactol monoacetate (11), was deduced from high resolution $^1$H NMR analysis and chemical degradation.[12] These alcohols are closely related to caespitol[13] and isocaespitol[14] isolated by Gonzales and coworkers. A brominated diterpene was also isolated and x-ray analysis[15] revealed it to have structure 12 (14-bromoobtus-1-ene-3,11-diol). The carbon skeleton of 12 was first observed in obtusadiol isolated by Howard and Fenical[16] from the red alga *Laurencia obtusa*.

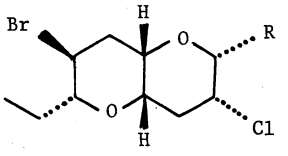
Further study of the Bimini *A. dactylomela* extracts has now led to the isolation of additional minor metabolites. One of these has been assigned[17] structure 13 on the basis of mass spectral data and comparison of its $^1$H NMR spectrum with that of related compounds, especially 10, 8-desoxy-isocaespitol (14)[18], and caespitol (15). The chemical shift and multiplicity of the -CHBr- proton in the ether ring of 13 matches closely that of the analogous protons in 10 and 14, while the positions of the methyl signals in 13 match those of 15 more closely than any other compound in the series, suggesting an axially oriented methyl group deshielded by a chlorine in 13. The chemical shift (4.71 ppm) and J values (4.7, 13) of the second downfield methine proton signal in 13 are very similar to that of the analogous proton in 11. This is consistent with structure 13 in which the axial tertiary hydroxyl group deshields the axial -CHBr- proton.

Four new, $C_{15}$ straight-chain halogenated ethers (16-19) belonging to the family of ethers represented by dactylyne (7) have also recently been isolated.[17] Spectral data clearly indicated that all have the terminal enyne feature. Structures 16 and 17 were among the possible ones deduced for two of the ethers from the results of extensive decoupling experiments at 270 MHz, but an unambiguous assignment of the relative position and stereochemistry of the heteroatoms could not be made. From single crystal x-ray diffraction analysis, structure 16 was confirmed for one of these ethers, including the absolute configuration depicted. Catalytic reduction of 16 and 17 gave the same octahydro product 20, thus establishing the complete stereochemistry for 17. Ether 17 is epimeric at C-12 with obtusenyne isolated by two different groups,[19,20] from algae. The 9-membered ring ether present in 16, 17, and obtusenyne is the largest encountered so far in this family of algal-derived $C_{15}$ straight-chain ethers.

X-ray diffraction was also used to elucidate structure 18 which contains two cis-fused tetrahydropyran rings, a unique feature in this family of $C_{15}$ ethers. The proposed structure of the isomeric ether 19 is based only on spectral data, the close correspondence of chemical shifts and multiplicities in the $^1$H NMR spectra of 18 and 19 strongly indicating that they differ only in the double bond geometry. Although many members in this family of $C_{15}$ ethers yield $^1$H NMR spectra exhibiting a variety of fairly well dispersed signals amenable to extensive decoupling experiments, unambiguous total structure assignments

are difficult to make solely on the basis of high resolution $^1H$ NMR data. The difficulty lies in making reliable chemical shift assignments in compounds which contain in close proximity a variety of heteroatoms that induce similar chemical shifts.




16 R = $-CH_2-CH=CH-C\equiv CH$(E)

17 R = $-CH_2-CH=CH-C\equiv CH$(Z)

18 R = $-CH_2-CH=CH-C\equiv CH$(E)

19 R = $-CH_2-CH=CH-C\equiv CH$(Z)

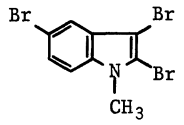
20 9,10-dihydro, R = n-$C_5H_{11}$

To date, sixteen new compounds have been isolated from *A. dactylomela* from Bimini. Work on isolation of other compounds is still in progress.

Since it is known that sea hares accumulate organics from their algal diet[2], we have examined extracts of one alga, as yet unidentified, found by gut analysis to be a major component of the Bimini *A. dactylomela's* diet at the time of collection. Thus far we have isolated[17] dactylyne (7) and the ether 17 from this alga.

Since *Aplysia* collected at Bimini yielded such a fascinating group of new metabolites, we were eager to analyze *Aplysia* from other locations as well. We were able to obtain a batch of *A. dactylomela* off La Parguera, Puerto Rico, and have found these to be a rich source of a totally different array of new and known compounds. The first metabolite isolated was the N-methylindole 21 which had been isolated by Rinehart and coworkers[21] from the alga *Laurencia brongniartii*. Several chamigrene derivatives were also isolated: elatol (22)[22], isoobtusol acetate (23) (the alcohol had been previously reported)[23], and the dibromotrienol (24)[24]. Along with 24 we isolated an isomeric dibromotrienol that we have assigned[25] the structure 25 by comparison of the $^1H$ NMR spectra of the acetates of 25 and 24. Isomers 24 and 25 interconvert to approximately 1:1 mixtures upon standing at room temperature for a couple of days and hence spectral data must be acquired promptly after isolation. This same type of isomerization has been reported[24] for the ketone 26 prepared by oxidation of 24.

In addition to these chamigrenes, a small amount of allolaurinterol acetate (27)[26], isomeric with both laurinterol[27] and isolaurinterol[28], was isolated.

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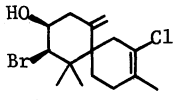


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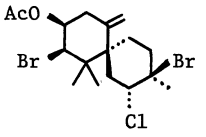


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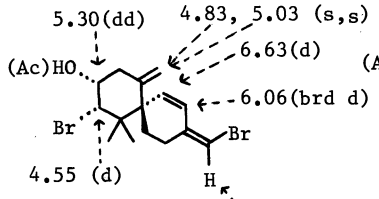


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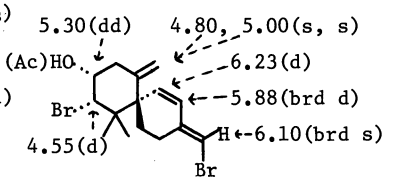


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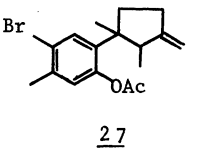
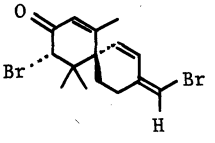
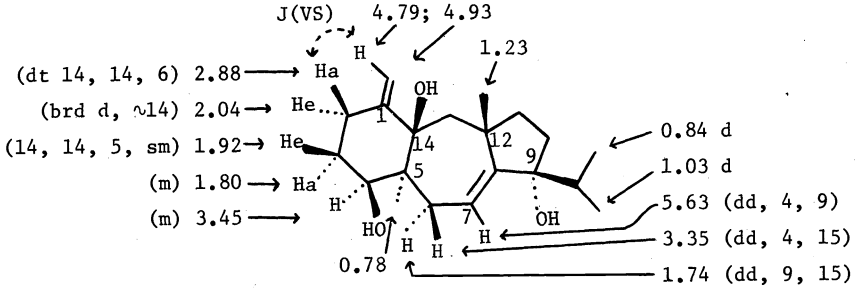



27

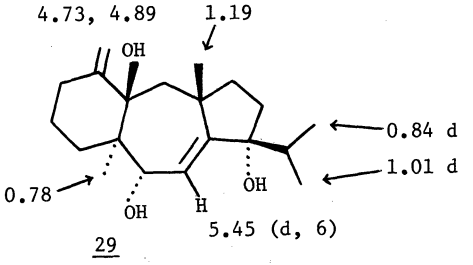



26

A variety of new diterpenes have also been obtained. The first of these (28) was found to have the formula $C_{20}H_{32}O_3$ by high resolution mass spectral analysis. $^1H$ NMR data for 28 resembled that reported[29] for dolatriol (29) in many respects and hence identical carbon skeletons were inferred. However, the isopropyl methine hydrogen in dolatriol was reported[29] to absorb at 2.6 ppm while that in 28 was found by decoupling to resonate at 1.95 ppm. Hence, it was of importance for confirming our structure to determine if the isopropyl group in 28 was attached to a tri- or a tetrasubstituted center. This question could be resolved by observing the multiplicity of the isopropyl methine hydrogen, but in the case of 28, this signal was overlapped by those of other hydrogens, and hence the multiplicity could not be directly observed. Conventional double irradiation of one of the enantiotopic isopropyl methyl signals resulted in a barely perceptible change in the complex absorption envelope at 1.95 ppm, see Fig. 1. However, using a recently described[30,31] technique designated as difference double resonance (DDR) it was possible to observe the decoupled signal of the isopropyl methine hydrogen free of the other overlapping signals, see Fig. 1. In this technique, two sequentially acquired FT spectra of equal intensity, one without and the other with decoupling, are subtracted from each other and the resultant difference spectrum is plotted. Ideally, all signals are nulled except those of protons affected by the decoupling. In the display method shown in Fig. 1, the decoupled signal from the isopropyl methine proton is observed as an inverted quartet at 1.95 ppm. Hence, the isopropyl group must be attached to a quaternary center as shown in 28, identical to the situation in 29.




28




29

With the aid of difference double resonance spectroscopy it was also shown that the olefinic proton at C-7 was coupled to a pair of methylene protons which in turn exhibited further obvious coupling only with each other. However, decoupling revealed that one of these methylene protons was W-coupled with the higher field quaternary methyl signal (0.78 ppm) as expected for the β-axial proton at C-6 in 28.

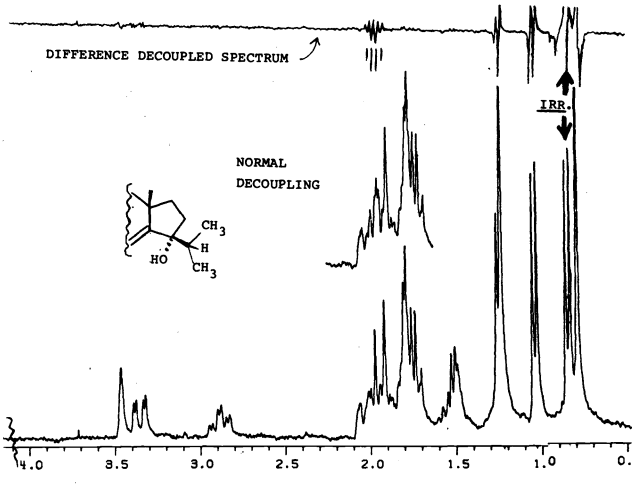


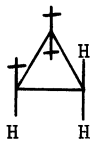

Fig. 1
Normal and difference double resonance decoupling of 28.

Only one signal (3.45 ppm) compatible with a methine proton deshielded by an OH group was present in the spectrum and this could be assigned to the C-4 equatorial hydrogen as a result of further DDR experiments. Thus, irradiation of one of the exomethylene signals demonstrated that the doubled triplet signal at 2.88 ppm was due to the axial proton at C-2, and this proton and the 2° alcohol methine proton (H-4) were found to be coupled to a common pair of protons (C-3 H's) in accordance with expectations for structure 28. The downfield position of the allylic 2-axial proton signal is due to 1,3-pseudodiaxial interactions between the proton and two hydroxyl groups.

The most interesting diterpenes isolated from the Puerto Rico *Aplysia* specimens are a group which have new carbon skeletons containing cyclopropane and cyclobutane rings. The first and most abundant of these was the acetoxytriol 30, designated parguerol[32] $C_{22}H_{33}O_5Br$ by high resolution ms and combustion analysis. Neither the natural product, nor its peracetate, perbenzoate or perphenylurethane derivatives have crystallized. IR and NMR data revealed that parguerol (30) contained two quaternary methyl groups, 1.06, 1.13 ppm, one acetate group (1725 $cm^{-1}$ ; 2.08 ppm), one double bond [trisubstituted; $^{13}C$ NMR 142.9 (s); 117.4 (d)], and one trisubstituted cyclopropane ring, see Fig. 2. These groups account for 3 degrees of unsaturation and confirm that parguerol must contain three more rings. Intense hydroxyl absorption in the infrared spectrum of 30 coupled with several downfield signals in the $^1H$ NMR spectrum indicated the presence of a variety of primary and seconday alcohol groups. Acetylation of 30 ($Ac_2O$, Py) resulted in the addition of three acetyl groups to give the tetraacetate 31, confirming that there were three hydroxyl groups in the natural product, and hence no ether links were present. Comparison of the $^1H$ NMR data for 30 and 31 led to an assignment of the partial structures containing hydroxyl and bromine shown in Fig. 2. The chemical shift changes indicated that bromine was attached to the secondary position of the +CHBr-$CH_2$-OH group.

| | PARGUEROL (30) | PARGUEROL ACETATE (31) |
|---|---|---|
| +$CH_2$OH | 3.38 d 11.5 | 3.71 |
| | 3.52 d 11.5 | 4.04 |
| Br<br>+CH-$CH_2$-OH | 3.84 dd 9,13 | 4.27 (Δδ 0.43) |
| ↑ | 3.95 dd 3.13 | 4.31 (Δδ 0.36) |
| └---------- | 4.26 dd 3,9 | 4.49 (Δδ 0.23) |
| -CH-OH | 3.12 brd dt (11,5) | 4.41 |

(30)




0.09 t 5
0.87 dd 5,10
1.04 m

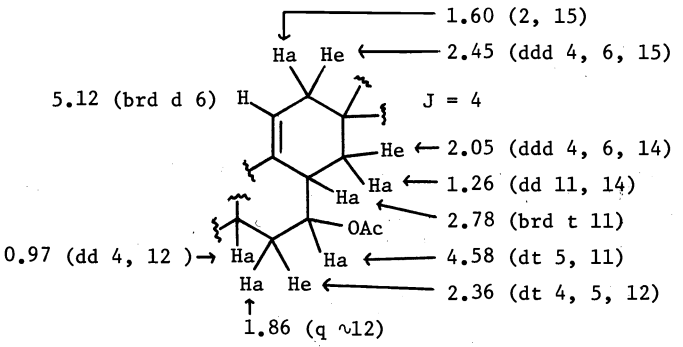



**Fig. 2 Partial structures deduced for parguerol from NMR data. Data in upper half of Fig. obtained in $CDCl_3$; lower half in $C_6D_6$.**

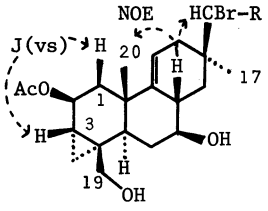
Some information regarding the juxtaposition of functional groups in Fig. 2 was obtained by microscale chemical conversions. Zinc-acetic acid treatment of parguerol gave several products, one of which contained an ABX absorption pattern in its $^1H$ NMR spectrum that was characteristic of an isolated vinyl group. Irradiation of the X signal of this group sharpened one of the quaternary methyl signals, thus inferring the partial structure -$C(CH_3)$-CHBr-$CH_2$-OH in parguerol.

A large segment of the carbon skeleton in 30 was deduced from extensive proton decoupling studies (270, 360 MHz in $C_6D_6$) of the acetate derivative 31. These studies revealed

the extended spin system corresponding to the partial structure shown in Fig. 2. Since the coupling constants were typical for vicinal axial/equatorial protons on cyclohexane/cyclohexene rings it was assumed that the unclosed ring in Fig. 2 was also a cyclohexane ring.

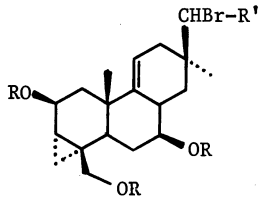
In the high resolution spectra of 31 and other derivatives in this series, sine bell resolution enhancement[33] of the FT acquired spectra aided immensely in making accurate measurements of J values and in resolving lines of partially overlapping signals.

A logical manner in which to combine all the above partial structures was suggested by reviewing known diterpene structures that contain a geminally situated methyl and ethyl group which would correspond to the $-C(CH_3)CHBr-CH_2OH$ unit in parguerol. Of these, the pimarane skeleton was found to accommodate all the structural elements found in parguerol by assuming that one of the ring A geminal dimethyl groups of pimarane was bonded to C-3 to form a cyclopropane ring. Thus structure 30 was suggested for parguerol. This structure with the relative stereochemistry shown is compatible with all the observed couplings. The cyclopropane ring helps account for the low field resonance (5.35 ppm) of the acetate deshielded methine proton in parguerol. Although the C-3 cyclopropane proton is not discernably coupled to the C-2 proton, it is W-coupled (very small J) to one of the protons (1.82 ppm) at C-1. The observation of a small W-coupling between H-1eq and H-3 requires that the latter also be equatorial, and hence the cyclopropane ring must be α-oriented. This was further confirmed by demonstrating a β-orientation for the hydroxymethyl group at C-4 by shift reagent studies. Thus, the angular methyl group at C-10 experienced a europium induced downfield shift of 2.5 ppm while the other quaternary methyl signal shifted only 0.1 ppm under the same conditions. The axial orientation of the acetoxy group at C-2 follows from the small couplings noted for the C-2 proton (5.48 ppm), and this was supported by solvent induced chemical shifts. Thus, removal of the acetyl group from 30 (LAH, R.T.) gave a tetrol, 32, whose quaternary methyl signals appeared at 1.04 and 1.20 ppm in $CDCl_3$, but which were shifted to 1.17 and 1.45 ppm in pyridine-$d_5$. Since the pyridine induced shifts[34] for the two methyl groups in 30 are only 0.15 and 0.19 ppm, the tetrol results support the 1,3-diaxial relationship of the acetate and the C-10 quaternary methylgroup in 30.




30 R = $CH_2OH$

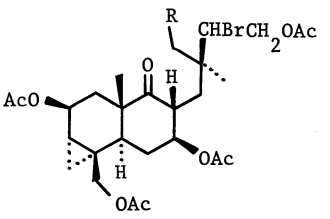
36 R = $CH_2OAc$




31 R = Ac, R'= $CH_2OAc$

32 R = H, R'= $CH_2OH$

33 R = H, R'= CHO




34 R = $CH(OMe)_2$

35 R = $CO_2H$

The relative configuration at C-13 was ascertained from NOE results, using the recently introduced difference NOE measurement.[30,31,35] As in the case of DDR, all signals are nulled in the difference NOE spectrum except those experiencing the NOE. The results depicted in Fig. 3 clearly show an NOE effect between the C-8 β-hydrogen and the C-20 and C-15 protons.

Additional supporting evidence for structure 30 was obtained from the following conversions. Manganese dioxide oxidation of 32 to probe for a possible allylic alcohol group gave a product assigned structure 33 ($C_{22}H_{31}O_5Br$, MS) that contained an aldehyde as the only carbonyl moiety (IR, $^1H$ NMR, MS). Decoupling confirmed that the aldehyde proton was coupled to the +CHBr- group. Ozonolysis of 31 in $CH_2Cl_2/CH_3OH$ followed by reduction of the ozonide with dimethyl sulfide yielded the dimethyl acetal 34. The acetal proton showed the expected triplet multiplicity, whereas the signal for the adjacent methylene group was a simple doublet rather than the more complex pattern possible from these diastereomeric protons. Oxidation of 34 with Jones reagent yielded the acid 35. The $^1H$ NMR spectrum of 35 in $C_6D_6$ was simpler than previous compounds in the series and the H-6, H-5 signals could be clearly observed, allowing verification of their multiplicities.

Two other cyclopropane-containing metabolites were obtained in minor amounts, both of which exhibited $^1H$ NMR spectra very similar to 30. In fact, the spectrum of the first of these, 36, was nearly identical to that of 30 with the exception that 36 contained two acetate methyl signals, and the multiplets corresponding to the $+CHBr-CH_2-OH$ group were observed at positions close to those recorded for peracetate 31. Hence, it was concluded that 36 is the 16-acetyl derivative of 30. Acetylation of 36 yielded a product whose 270 MHz $^1H$ NMR is identical to that of 31.

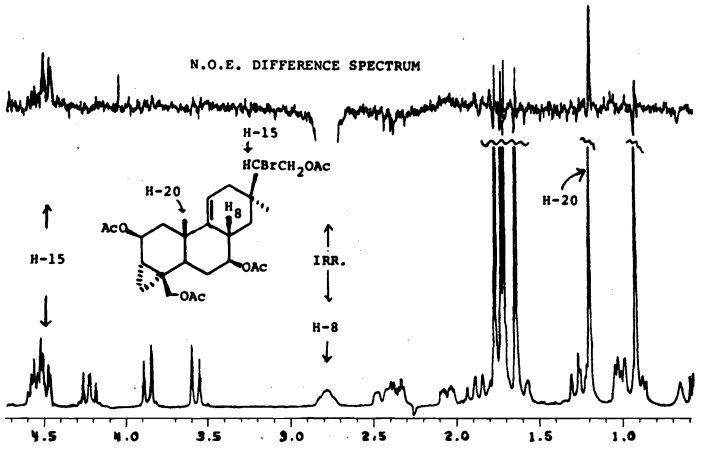



Fig. 3 NOE difference spectrum of parguerol acetate(31).

The third cyclopropane-containing metabolite, 37, a monoacetate, was acetylated to facilitate purification. The $^1$H NMR spectrum of the resulting triacetate 38 was similar in most respects to that of 31 but with two notable exceptions. First, the AB quartet signals corresponding to the $+CH_2$-OAc group in 31 were missing in the spectra of 38 (and 37), and a new methyl singlet was evident at 1.00 ppm. Also, the three cyclopropyl hydrogen signals in 38 were all shifted upfield relative to those in 30 and 31. These differences are consistent with structure 37 for this minor metabolite.

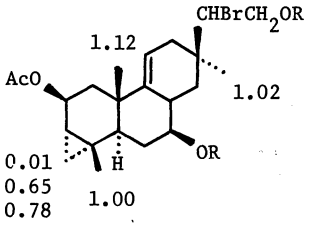
Two additional bromoditerpenoids were obtained that seemed clearly related to 30, 36, and 37 as judged from $^1$H NMR analysis, although they did not contain cyclopropane hydrogen signals. The first of these, designated isoparguerol (39), $C_{22}H_{33}O_5Br$, was determined to be a monoacetate (1725 $cm^{-1}$; 2.03 ppm, s), but upon routine acetylation yielded only a triacetate (40) which still retained hydroxyl absorption in the infrared spectrum indicating that one $3^o$ hydroxyl group was present. The $^1$H NMR spectrum of the triacetate 40 in $C_6D_6$ was nearly identical in many respects to those of 30, 36, and 37, and decoupling data provided evidence that the B/C rings and their associated substituents were identical in all these compounds.

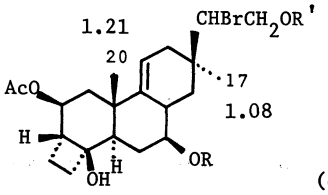
Obviously missing from the spectrum of 39 in addition to the cyclopropane proton signals, were the AB quartet resonances for the isolated hydroxymethyl group. A chemically logical explanation that would account for the loss of cyclopropane and $+CH_2$-OH groups with concurrent generation of a new $3^o$ hydroxyl group but no new methyl group, would be rearrangement of the cyclopropylcarbinyl alcohol to a tertiary cyclobutyl alcohol moiety. This hypothesis was confirmed by decoupling and shift reagent experiments on the acetate 40. Difference double resonance decoupling was particularly useful in identifying the position and multiplicity of the cyclobutane protons specified on partial formula 41. In the presence of europium the signal for the C-20 protons was shifted downfield much farther than that for the C-17 protons ($\Delta\delta$ 0.65 vs. 0.16), indicating that the hydroxyl group at C-4 is $\beta$-oriented. An A/B trans-ring fusion was assigned on the basis of the $\sim$8 Hz couplings observed for the C-5 proton with each of the C-6 methylene protons. Thus, the overall structure 39 is proposed for isoparguerol.

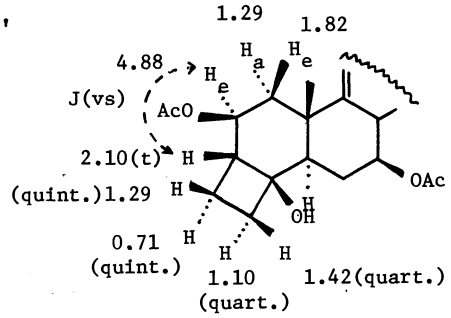
In the mass spectrum of isoparguerol, sets of fragment ions of significant intensity are observed which correspond to the loss of $CH_2{=}CH_2$ from precursor ions as might be expected for the cyclobutyl structure. The same fragment ions are not observed in the case of 30, 36, or 37.

A second cyclobutane containing metabolite which has been assigned the structure 42 was also isolated. Acetylation of compound 42 gave the triacetate 40 thus providing a correlation with alcohol 39. $^1$H NMR data confirmed the position of the second acetate group in 42.

Overall, 12 compounds, most having new structures, have now been isolated from *A. dactylomela* collected near La Parguera, Puerto Rico. Thus far, not one compound has been found in common between the *Aplysia dactylomelas* collected from these two different locations.



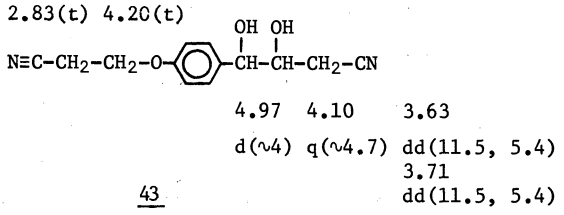

37 R = H
38 R = Ac

39 R = H, R' = H
40 R = Ac, R' = Ac
42 R = H, R' = Ac

41

Another sea hare, *Bursatella leachii pleii* (Rang), also collected near La Parguera, Puerto Rico, but from a habitat quite different from that occupied by *A. dactylomela*, has also been studied. The digestive glands, usually the richest source of novel metabolites, were found to contain little other than aliphatic lipid materials and sterols. However, from extracts of the remainder of the animal, a novel dinitrile, bursatellin (43), was isolated. High resolution mass spectral data yielded the molecular formula $C_{13}H_{14}N_2O_3$ and IR data indicated the presence of hydroxyl and nitrile groups. Upon acetylation, bursatellin afforded a diacetate whose infrared spectrum possessed nitrile and acetate absorption but no residual hydroxyl absorption. Hence the third oxygen in bursatellin was assigned to an ether group. $^1$H NMR and mass spectral data provided the basis for assigning structure 43.[36] In confirmation of this structure, it was found that bursatellin underwent oxidation with $MnO_2$ to give the expected hydroxybenzophenone and gave a positive test for 1,2-diols with periodate. The possibility that bursatellin might contain isonitrile groups, as have been found in a number of other marine natural products[37], was ruled out by the absence of $^{14}$N-$^1$H coupling characteristic of isonitriles[38].

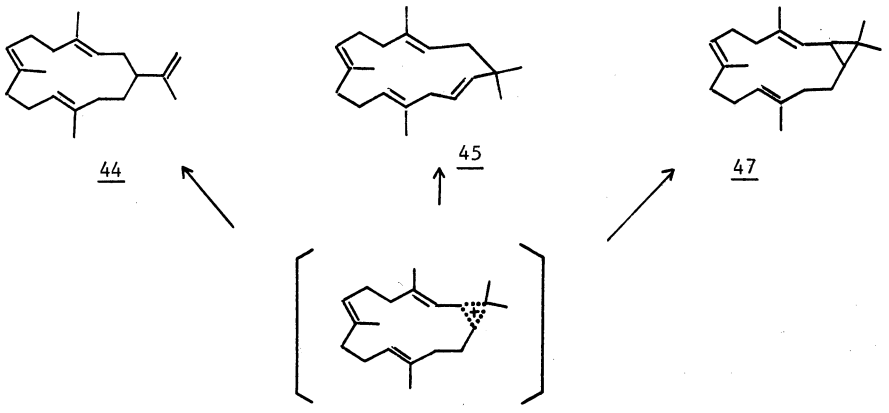
Although the chemistry of *B. leachii pleii* is not nearly as varied and interesting as that of *Aplysia*, our work overall verifies that opisthobranch molluscs are generally excellent sources of novel metabolites.




43

## COELENTERATES

Although this paper is concerned primarily with Caribbean marine organisms, we would like to include an observation from studies of a Pacific soft coral, *Sinularia conferta*, that has significance in terms of biosynthesis in coelenterates. Interestingly, while cembranolides are common metabolites from gorgonians, the corresponding parent hydrocarbon, i.e. cembrene or isomers thereof, have not been reported from these animals. In contrast, cembrene-A (44) and also the biogenetically related hydrocarbon flexibilene (45) have been isolated from soft corals or alcyonaceans.[34] Cembrene and flexibilene are considered to arise by cyclization of geranylgeranyl pyrophosphate between C-1 and either C-14 or C-15, respectively, with the cyclopropyl cation (46) being invoked as a possible intermediate.[39] Carbocation (46) is also a logical precursor for casbene (47) which to date has only been isolated as a trace metabolite from biosynthetic experiments using an enzyme preparation from castor bean seedlings[40], and not from any intact organisms. In an investigation of *Sinularia conferta* we found[41] cembrene-A and flexibilene to be major hydrocarbon metabolites, but during the careful chromatography needed to separate these isomers, a minor hydrocarbon component was isolated which was identified as casbene by mass spectral and $^1$H NMR analysis. This is the first isolation of casbene from an intact organism. Furthermore, this documents the co-occurrence in a single organism of hydrocarbons representing all three carbon skeletons anticipated from the hypothetical intermediate (46). In view of the wide variety of oxygenated forms of cembrene already isolated from alcyonarians and alcyonaceans, it seems likely that future work will uncover oxygenated forms of flexibilene and casbene.



44 45 47

46

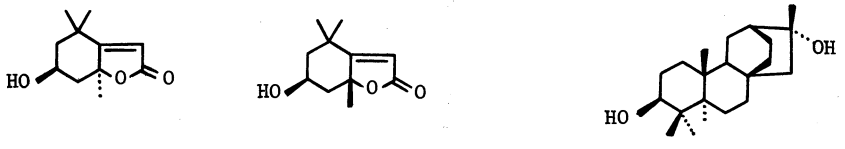
SPONGES

The sponge *Tedania ignis*, colloquially known as the fire sponge[42], is common in the Caribbean in shallow waters and on mangrove roots. This sponge is reputed to cause upon contact a dermal inflammation[42,43] believed by some to be chemically induced and by others to be due to spicule embedding. We found extracts of this sponge to be cytotoxic and hence we initiated a bioassay-guided search for the active components. The most active cytotoxic components still elude us, but a number of interesting metabolites have been isolated in the course of this investigation, for example the cyclic dipeptides cyclo-L-prolyl-L-valine, cyclo-L-prolyl-L-leucine, cyclo-prolyl-alanine. It is interesting to note that these cyclic dipeptides have also been isolated[44] from fungi, yeasts and bacteria. Indeed, diketopiperazines in general are common metabolites of these microorganisms.[44] The occurrence of these cyclic dipeptides in the sponge extract thus reinforces the speculation[45] that some metabolites isolated from sponges may originate from ingested or symbiont microorganisms.

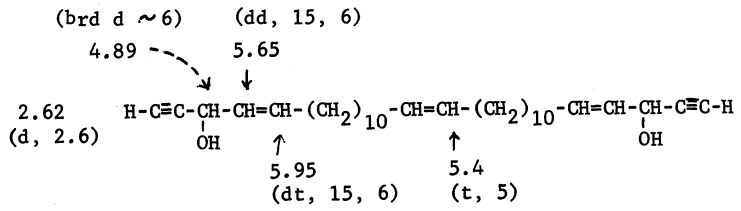
Also isolated from *T. ignis* was epiloliolide (48)[46], likely a carotenoid oxidation product. This lactone appears not to have been isolated previously from natural sources, although the epimeric lactone, loliolide (49), has been identified in fourteen different plant species. Recently, loliolide was isolated[47] from a sea hare *Dolabella ecaudata* collected in the Indian Ocean. Interestingly, that isolation also resulted from a bioassay-guided search for cytotoxic compounds. Epiloliolide was found[48] to be inactive in the KB bioassay at 10 mcg/ml while loliolide is reported[47] to exhibit an $ED_{50}$ of 10 mcg/ml.

A trace amount of a new diterpene diol, atisane-3β,16α-diol (50) was also isolated from *T. ignis*. Its structure was secured by x-ray analysis.[48] The CD curve of the derived 3-ketone showed a negative Cotton effect similar to that observed for 4,4-dimethylcholestan-3-one and hence the new alcohol has the absolute configuration depicted in formula 50.

From a *Xestospongia* sp. of sponge collected off La Parguera, Puerto Rico, we have isolated[49] a novel diacetylenic lipid, tentatively assigned structure 51. This lipid, $C_{32}H_{52}O_2$ by high resolution mass spectrometry, formed a diacetate whose infrared spectrum still showed sharp, strong absorption characteristic of an acetylenic proton (3360 $cm^{-1}$), but extremely weak triple bond absorption at 2110 $cm^{-1}$. The $^1H$ NMR spectrum showed a sharp doublet at 2.62 (J = 2.6) ppm attributable to a terminal acetylenic proton. Acetylenic functionality was confirmed when it was found that the product obtained from catalytic hydrogenation of the unsaturated lipid was devoid of both the 3360 $cm^{-1}$ IR band and the 2.62 ppm doublet in the $^1H$ NMR spectrum. From decoupling results the terminal portions of structure 51 were deduced. A highly symmetrical structure was suggested by the exact coincidence in chemical shift of all the like downfield protons. Analysis of the high resolution mass spectral data supported this view, leading to tentative assignment of the isolated double bond to the central location in 51. It is interesting to note that we have previously isolated a brominated C-16 acid containing an acetylenic linkage from a related sponge, *Xestospongia muta*.[50]



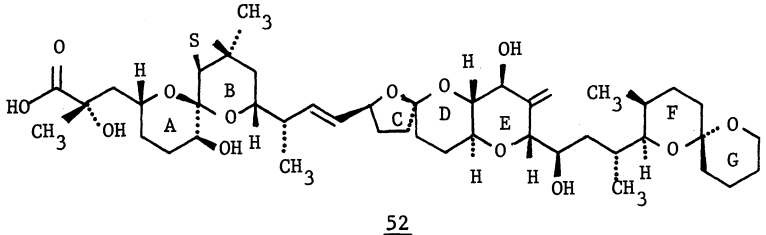
48 49 50




51

As mentioned earlier, it is speculated[45] that bacteria present in marine sponges as symbionts or as a result of filter-feeding by sponges, may produce some of the metabolites isolated from the entire sponge assemblage. Confirmation of the bacterial origin of a specific metabolite would normally be difficult, unless perhaps that product were clearly unique to certain microorganisms. Recently, we have isolated just such a unique metabolite from the sponge *Pandaros acanthifolium*. This metabolite was a trace component present in the highly colored, typical aliphatic lipid portion of the sponge extract. Since it is a trace component, it most likely would have gone undetected by routine tlc and $^1$H NMR scanning for interesting metabolites, because even purified fractions yielded spectra indicative only of saturated/unsaturated acids or alcohols. Only due to its outstanding activity in the cytotoxicity bioassay were we directed to it. And even the indication of cytotoxicity did not arouse great interest during fractionation because it is known[51] that oleic and palmitoleic acids exhibit $ED_{50}$ values of 0.67 and 0.96 mcg/ml against the P388 cell line. Only after repeated silica gel chromatographies were we rewarded with an interesting $^1$H NMR spectrum indicative of an unusual structure.

Inadequate amounts of material were initially available for combustion analysis. Mass spectral analysis, both electron impact and field desorption gave poor, non-definitive results. Fortunately, acanthifolicin could be crystallized and x-ray analysis revealed it to have structure 52.[52] Acanthifolicin is thus a novel member of the polyether class of antibiotics, which heretofore have only been isolated from bacteria.[53] The most unique feature in the structure of acanthifolicin, the first carboxylic acid ionophore reported[54] from marine sources, is the episulfide group. This functionality is rare in any natural product, and is an unprecedented feature among the known polyether antibiotics.[53] Although no experimental evidence has been obtained to prove that acanthifolicin is of bacterial origin, we think the isolation of this exclusively bacterial type of metabolite from a sponge extract supports the belief[45] that bacteria are the sources of some of the products isolated from sponges.




52

Only the relative configuration shown in formula 52 has thus far been established. In addition to its unique episulfide functionality acanthifolicin is distinguished by having the longest carbon backbone ($C_{38}$) of any reported polyether antibiotic. It also has less alkyl branching than is found in many polyether antibiotics: 6 methyl groups in a $C_{38}$ backbone compared to an average of 10-12 methyl/ethyl groups in carboxylic acid ionophores with $C_{25-30}$ backbones. Acanthifolicin does not have a hydroxyl group near the tail end of its carbon backbone as is common in most other polyether antibiotics, and which normally is the site for hydrogen bonding of the carboxyl group to form a macrocyclic cavity. Instead the carboxyl group hydrogen bonds in the crystal structure to the hydroxyl group on ring E to form this cavity. Ten of the thirteen oxygen atoms in the molecule cluster around this 5-7 Å diameter cavity, with seven oxygen atoms within 4.0 Å from its center point, a situation quite suitable for complexation of a cation. Rings F and G extend away from this cavity region giving the overall molecule in this crystal form a length of about 15 Å. There are two short intermolecular O.....O distances suggesting hydrogen bonding: O(1).....O(10), 2.93 Å, and O(3).....O(4), 2.79 Å. Acanthifolicin has two spiro-fused six-membered ketal ring systems whereas spiro ketal groups in other polyether antibiotics usually involve six- and five-membered rings. The presence of two fused tetrahydropyran rings (D, E) is also unique among the known natural carboxylic acid ionophores.

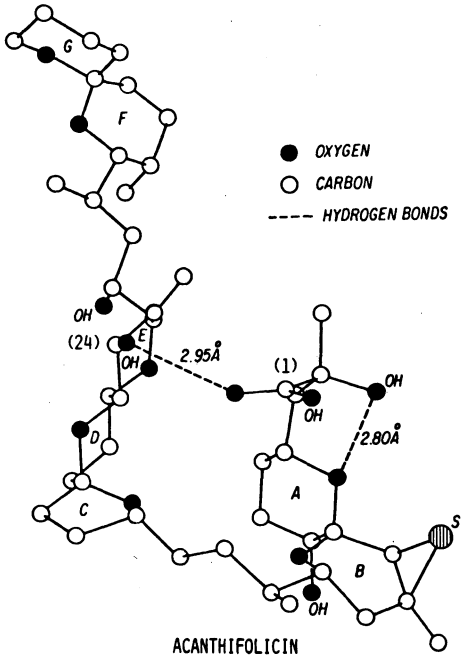



Fig. 4 Stereoformula of crystalline acanthifolicin.

Acanthifolicin exhibits $ED_{50}$'s of 2.08 X $10^{-4}$, 2.1 X $10^{-3}$, and 3.9 X $10^{-3}$ mcg/ml, respectively, against P388, KB, and L1210 cell cultures. In *in vivo* tests 52 has proven to be toxic down to 0.14 mg/kg doses and testing at lower doses has not been completed as yet.

Approximately sixty carboxylic acid ionophores have been reported to date, nearly all of which have been isolated from *Streptomyces* sp. of bacteria.[53] A few of these ionophores are commercially important as anticoccidial agents. We are in the process of evaluating acanthifolicin for this type of activity. Efforts are also underway to culture bacteria associated with *P. acanthifolium* that may produce acanthifolicin. In this connection it may be noted that a macrolide polyether containing boron, aplasmomycin, has been isolated[55] from a marine bacterium.

Work in progress in our laboratory suggests that other polyether antibiotics may be isolated from marine organisms by careful examination of the lipid fractions consisting predominantly of aliphatic components. We consider it likely that a family of these polyethers may emerge from future work with marine organisms.

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