Tetrahedron Vol. 38, No. 13, pp. 1881 to 1890, 1982
Printed in Great Britain.

0040-4020/82/131881-10$03.00/0
Pergamon Press Ltd.

CHEMICAL STUDIES OF MARINE INVERTEBRATES—XLV[1]

THE CHEMISTRY OF THREE NORSESTERTERPENE PEROXIDES FROM THE SPONGE *SIGMOSCEPTRELLA LAEVIS*[2]

M. ALBERICCI, J. C. BRAEKMAN,[3] D. DALOZE and B. TURSCH
Collectif de Bio-écologie, Faculté des Sciences, Université Libre de Bruxelles, 1050 Brussels, Belgium

(*Received in U.S.A. 22 June* 1981)

Abstract—The structures of sigmosceptrellin-A, -B and -C, three norsesterterpenes constituting the ichthyotoxic fraction of the sponge *Sigmosceptrella laevis* are represented by formulae **1a**, **2a** and **3a**.

The phylum Porifera, comprising the most primitive multicellular animals, contains some 5000 described species, most of them marine. These sessile organisms have world-wide distribution and are well represented in coral reefs where they often make an important contribution to the biomass and could potentially constitute an interesting food resource. Some sponges seem to derive protection from the presence of sharp spicules or tough fibrous components; others are cryptic and concealed into crevices or under rocks or coral slabs. Many species, however, grow exposed and are devoid of physical means of defence. It has been repeatedly suggested[4-7] that they are protected from predation by toxic or noxious chemicals, i.e. allomones. Only a few highly specialized fishes select these sponges as their main diet.[8]

Recent studies[4,6] have shown that in tropical waters 60 to 75% of the exposed sponges are toxic to goldfish in laboratory tests. Along the North American Western coast, the toxicity of sponges is inversely related to latitude[4,5] an observation explained by the increased species diversity in tropical shallow water fish, resulting in a more intense food competition and hence the natural selection of chemically protected species.

Although a great number and variety of secondary metabolites have been isolated from both toxic and non toxic sponges (see reviews by Minale[9] and Minale *et al.*[10]), few data have been published concerning the ecological role of these compounds.

Recently, however, several authors[11-17] have reported the toxicity or antifeeding activity of sponge metabolites. Thus, halitoxin, a mixture of quaternary pyridinium salts isolated from three different *Haliclona* species,[11] is toxic to goldfish at 100 μg/ml. The sesquiterpene 9-isocyanopupukeanane, from *Hymeniacidon* sp. is lethal to fish,[13] whereas isodysidenin, an hexachlorinated metabolite isolated from *Dysidea herbacea*, is toxic to *Lebistes reticulatus* at 5 mg/l.[16] When tested on *Gambusia affinis* the toxic fraction of *Latrunculia magnifica* exhibits a LD_{50} of 0.4 mg/l.[18] The latrunculins were isolated from that fraction,[17] but no bioassay have been reported for the pure compounds.

Walker *et al.*[12] have recently shown that several sesterterpenes isolated from *Spongia idia* are toxic to other forms of marine life. For example, idiadione and 12-deacetyl-12,18-diepiscalaradial are toxic to the sea star *Pisaster giganteus* at a concentration of 5 mg/l and to the brine shrimp *Artemia* sp. at 10 mg/l. Both compounds immobilize the larvae of the red abalone *Haliothis rufescens* at 1 mg/l in sea water, whereas heteronemin is toxic to the gametes of the giant kelp *Macrocystis pyrifera*.[12]

Finally, two furanosesquiterpenes, the nakafurans-8 and -9, isolated by Schulte *et al.*[15] from *Dysidea fragilis* were shown to display antifeeding activity against two common reef fishes (*Chaetodon* spp.).

It is interesting to note that some sponges that produce allomones are nevertheless eaten by specific nudibranch mollusks. This is the case for the sponge *Hymeniacidon* sp. and its predator the nudibranch *Phyllidia varicosa*.[13,14] It has been stated that "the mollusk accumulates the allomone, which is the active constituent of its mucous skin secretion that is lethal to fish and crustaceans".[14] The antifeedant nakafurans-8 and -9, have been isolated from both the sponge *Dysidea fragilis* and its predators, the nudibranchs *Hypselodoris godeffroyana* and *Chromodoris maridadilus*.[15] Finally, the dorid nudibranch *Cadlina marginata*, the only predator observed to feed on *Spongia idia*,[12] contains besides other sponge metabolites, the sesterterpene idiadione, which is toxic to several marine invertebrates (*vide supra*). These data strongly suggest that some nudibranchs are able to store sponge allomones and to use them for their own defense against predators (e.g. fishes). A similar grazer-prey relationship has also been demonstrated between mollusks and algae.[19]

Many sponges living in tropical waters show antimicrobial activity. Burkholder, who summarized the earlier literature on marine antibiotics,[20] tested 20 species of Puerto-Rican sponges against 11 representative marine bacteria.[21] Two sensitive vitamin-requiring bacteria were inhibited by 12 and 15 sponge species respectively (60 to 75%), whereas the nine other were inhibited by 4 to 11 kinds of sponges (20 to 55%). More recently, Rinehart *et al.*[22] reported that 37% of the Baja California species (71 examined) and 82% of the Caribbean species (187 examined) show antimicrobial activity. However, it must be pointed out that the highest figures reported above are maximum ones taking into account a possible non-random selection of the species. Furthermore, number of extracts active on shipboard were found to be inactive in the secondary pharmaceutical screens.[22] This was attributed to the lower concentrations used in the secondary screens and the decomposition of the active compounds during storage.

In recent years, number of novel antimicrobial substances have been reported from Porifera.[23-34] These include terpenoids (e.g. puupehenone,[23] acanthellin-1,[24]

manoalide,[28] siphonodictval-A and -B),[32] polyphenols[26] lipid derivatives (plakortin[30] and related peroxides[34]), an isoquinoline derivative, renierone,[29] as well as derivatives of guanidine (the acarnidines[27]) and tryptamine (5,6-dibromo-N,N-dimethyltryptamine[25]). However, their antimicrobial activity (generally tested *in vitro* against terrestrial bacteria or yeasts) is not of the same order of magnitude than that of antibiotics derived from terrestrial microorganisms. Furthermore, the ecological functions of these sponge antibiotics is far from being clear. Burkholder[20] hypothesized that "in sponges which produce powerful antimicrobial substances, these substances may serve to inactivate microbial prey, as a preliminary to their digestion and use as food". Alternatively, these antibiotics, which do not harm the symbiotic bacteria, can be viewed as protecting the sponge from invasions by external pathogens.[28]

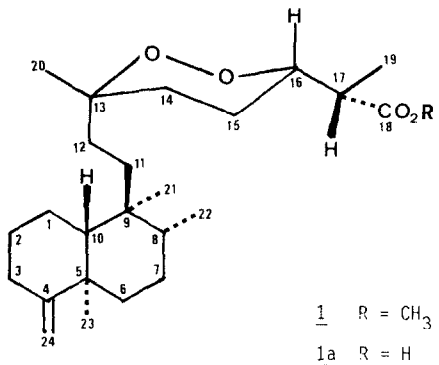
Quite recently, it has been suggested by Faulkner[7] that in temperate sponge-dominated assemblages there is a negative correlation between antimicrobial activity of the metabolites and the degree to which these sponges are fouled by epibionts. Many other sessile marine organisms also exhibit resistance to fouling, e.g. gorgonians,[20] algae,[20,35] bryozoans[35] and tunicates,[36] but the precise mechanism of this phenomenon has generally not been unambiguously demonstrated.

Another intriguing problem is the origin of the metabolites so far reported from sponges. Indeed, many demosponges and calcareous sponges are associated to different types of microorganisms, principally Cyanophyceae and bacteria.[37] The latter, which were shown to constitute 38% of the tissue volume in two mediterranean *Verongia* sp., may consist of five main morphological types in the same sponge.[38] It is possible that these microorganisms could be the source of some of the metabolites so far reported as isolated from sponges. In some cases the peculiar structure of the compounds strongly suggests that they originate from microorganisms (e.g. the polyether antibiotics acanthifolicin[39] and okadaic acid[40] and the thiazole derivatives dysidenin[41] and isodysidenin[16]).

It is also worth mentioning that some Porifera are able to prevent the growth of their neighbours, probably through the use of allomones. For example, *Siphonodictyon coralliphagum* is a burrowing sponge generally found in a living coral head where it maintains 5–10 mm zone of dead coral polyps around the base of the oscular chimney.[32] Although two antimicrobial phenolic aldehydes, siphonodictyal-A and -B have been isolated from the mucus of the sponge, their effects on the growth of the coral polyps was not determined. On the other hand, experiments with isodysidenin[16] have shown that topical application of this compound causes local necrosis in various sponges and corals.[42]

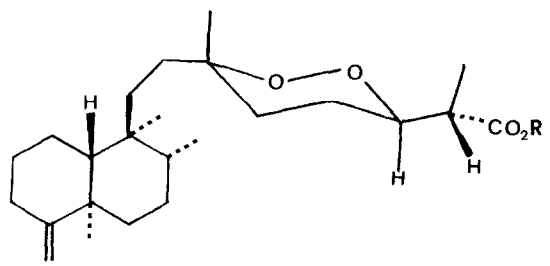
All the data gathered in this brief review suggests that secondary metabolites play a fundamental role in sponge survival and adaptation. However, much more biological and ecological work is needed before an exact appraisal of the chemically mediated interaction of sponges with their environment could be made.

Sigmosceptrella laevis is one of the numerous sponges living in the vicinity of Laing Island (Papua-New Guinea) that seem to suffer little if any predation. The dichloromethane extract of sun-dried specimens of this sponge is toxic to *Lebistes reticulatus* (LD: 25 mg/l). This toxicity is associated with an acidic fraction (LD: 5 mg/l) which, on methylation with CH_2N_2 followed by chromatography on silica gel, yielded three closely related compounds: sigmosceptrellin-A (**1**), sigmosceptrellin-B (**2**) and sigmosceptrellin-C (**3**) methyl esters. The methyl esters **1** and **2**, together with the methyl ketone **4** were also isolated from the crude extract.



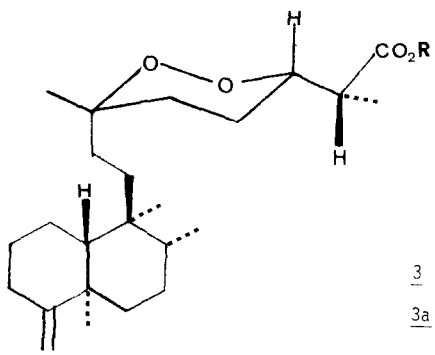
1 R = CH_3

1a R = H



2 R = CH_3

2a R = H



3 R = CH_3

3a R = H

Table 1. Comparison of the spectral properties of sigmosceptrellin-A, -B and -C methyl esters

	(1)	(2)	(3)
Molecular weight	406	406	406
Empirical formula	$C_{25}H_{42}O_4$ *	$C_{25}H_{42}O_4$	$C_{25}H_{42}O_4$
$[\alpha]_D$	+61.2°	−53.4°	+42.2°
^1H NMR			
HC-16	4.23 m	4.14 m	4.15 m
HC-17	2.56 quintet (7 Hz)	2.65 quintet (7 Hz)	2.59 quintet (7 Hz)
H_3C-19	1.10 d (7 Hz)	1.21 d (7 Hz)	1.22 d (7 Hz)
H_3C-20	1.04 s	1.23 s	1.09 s
H_3C-21	0.74 s	0.73 s	0.75 s
H_3C-22	0.81 d (5 Hz)	0.80 d (5 Hz)	0.81 d (5 Hz)
H_3C-23	1.03 s	1.03 s	1.05 s
H_2=C-24	4.51 bs	4.50 bs	4.54 bs
$COOCH_3$	3.67 s	3.70 s	3.71 s

* Established by HRMS.

In a previous communication[43] we reported the structure and relative configuration of sigmosceptrellin-A methyl ester (**1**) as deduced from X-ray diffraction analysis. Comparison of the spectral properties of esters **1**, **2** and **3** (Table 1) suggests that these compounds are stereoisomers. In particular their ^1H NMR spectra are almost identical except for some signals associated with the substituents of the 1,2-dioxane ring, suggesting that the stereochemical changes are confined to that part of the molecule.

To support this hypothesis, ester **2** was hydrogenated to the tetrahydroderivative **10** which was then oxidized either to the α,β-unsaturated ester **8** (Jones oxidation) or to the γ-lactone **9** (PCC or Ratcliffe oxidation). These two derivatives were found to be identical in all respects with those obtained previously from ester **1**, using the same degradation scheme[43] (Figure 1). Nevertheless, this correlation is not completely unambiguous since the catalytic hydrogenation of **1** and **2** to the tetrahydroderivatives **7** and **10** respectively is not stereospecific. Indeed GC analysis of these diols and of the oxidized derivatives **8** and **9** shows that we are dealing with a 9:1 mixture of the epimers at C-4. Examination of a Dreiding model of **1** clearly suggests that the C-4α epimer will be preferentially formed. To avoid this situation, a modified correlation scheme was devised. Cleavage of the $\Delta^{4(24)}$-exomethylene functions of **1** and **2** ($OsO_4/NaIO_4$) afforded ketones **5** and **13** respectively, which on catalytic hydrogenation followed by Jones oxidation, yielded the same α,β-unsaturated ester **12**, homogeneous by GC (Fig. 1). These results imply that sigmosceptrellin -A and -B differ by their configuration at C-16 and/or C-17.

Selective $LiAlH_4$ reduction of the carbomethoxy group of **1** followed by oxidative cleavage of the exomethylene group led to the keto alcohol **16**. Mesylation of the primary alcohol and hydrogenolysis ($Zn/C_2H_5OH/AcOH$) of the mesylate via the iodide, yielded the ketodiol **18** ($[\alpha] = -1.5°$). When this sequence of reactions was carried out, starting from **2**, the ketodiol epimeric at C-16 **22** ($[\alpha] = -22.3°$) was obtained, indicating that the esters **1** and **2** are either epimeric at C-16 or diastereoisomeric at both C-16 and C-17 (Fig. 2).

The following correlations demonstrate the correctness of the first hypothesis (Fig. 3). Treatment of the diacetate **27** (obtained by $LiAlH_4$ reduction of **1** into triol **26**, followed by acetylation) with oxalic acid afforded a mixture of dehydro compounds (as shown by GC and ^1H NMR), which without purification was oxidized with $OsO_4/NaIO_4$. Two compounds were isolated from the resulting mixture: the methyl ketone **6** arising from cleavage of the Δ^{13} double bond, and the diketone **28**, coming from cleavage of the $\Delta^{13(20)}$-exomethylene. The methyl ketone **6** was found to be identical in all respects with the derivative obtained by $OsO_4/NaIO_4$ cleavage of the $\Delta^{4(24)}$-exomethylene function of natural compound **4**. Methanolysis (K_2CO_3/MeOH) of the acetate groups of **28** afforded keto diol **29** which on selective protection of the primary alcohol with dimethyltertiobutylsilyl chloride[44] and oxidation of the secondary alcohol with pyridinium dichromate[45] gave the expected triketoderivative **32**. The latter was also obtained when the same degradation scheme was applied to ester **2**. This implies that sigmosceptrellin-B is the C-16 epimer of sigmosceptrellin-A.

It is interesting to point out that, on attempted purification by silica gel chromatography, diol **29** is partially transformed into a less polar derivative having lost a molecule of water (M^+ at 348). Moreover, the IR spectrum shows an important decrease in the intensity of the carbonyl band at 1710 cm^{-1} and the absence of an hydroxyl band at 3500 cm^{-1}. The ^1H NMR spectrum, besides the signals expected for the bicyclodecanone ring, shows three signals of 1 H each at 4.17, 4.01 and 3.48 ppm respectively, attributable to three protons adjacent to an oxygen atom and a 3H doublet at 1.25 ppm

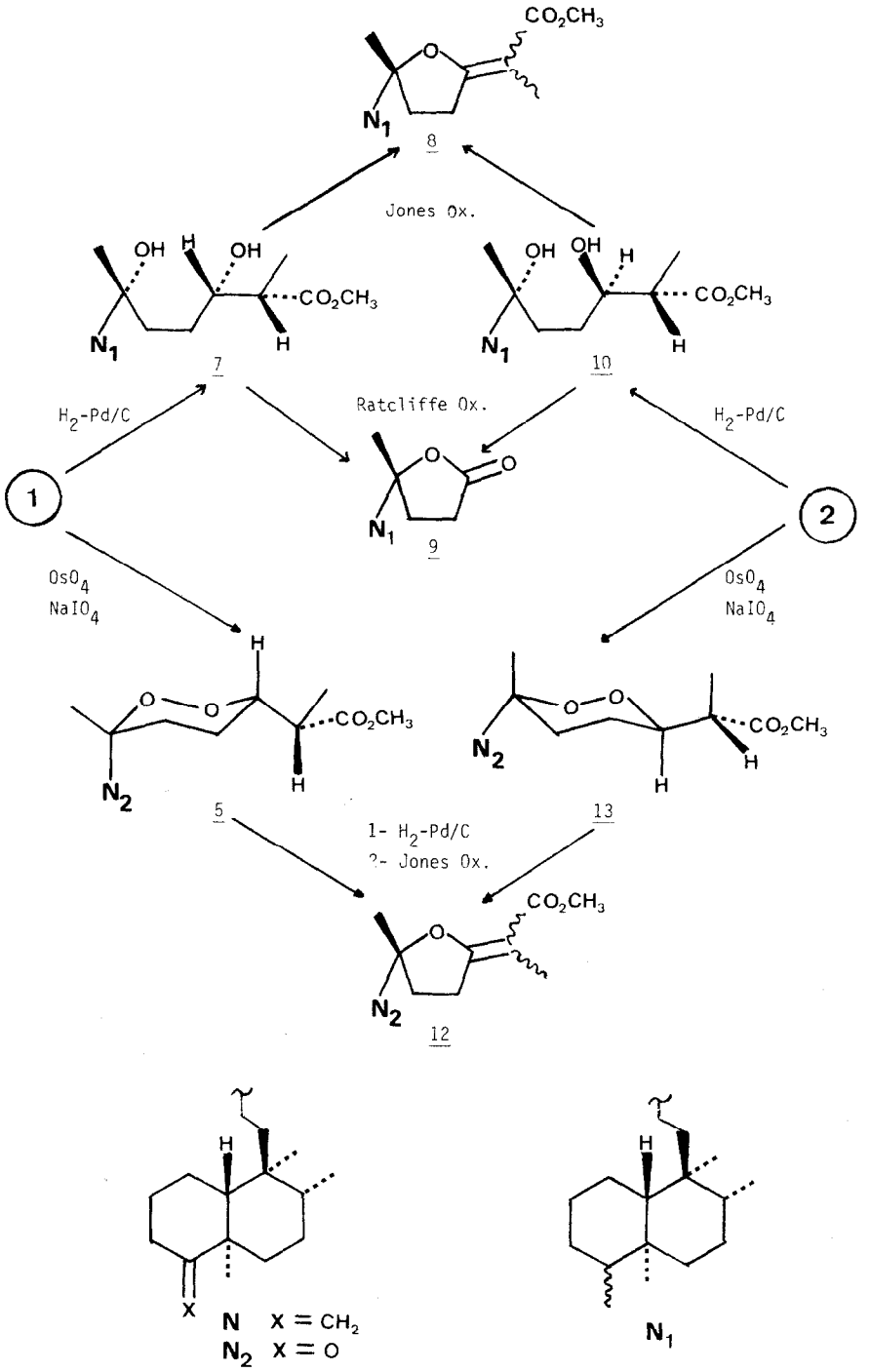


Fig. 1.

attributable to the $^{19}CH_3$ (Table 2). The only hypothesis that fits all these data is that a ketalization reaction on **29** catalyzed by silica gel has taken place, leading to the formation of ketal **30**. This hypothesis is supported (1) by the fact that on treatment with a mild acidic solution the starting diol **35** is partially recovered, (2) by the coupling constants observed for the H_2C-18 protons which are in good agreement with those expected for a *cis*-2,5-dialkyl-1,3-dioxane moiety.[46] The same type of rearrangement which leads to the ketal **37** is observed on attempted purification of the diol **36** originating from sigmosceptrellin-B methyl ester. Again, the observed coupling constants for the H_2C-18 protons are in good agreement with the predicted values. Moreover, comparison of the chemical shifts of the $^{19}CH_3$ in **30** and **37** shows that this Me undergoes an important downfield shift in the former (Table 2). This may be attributed to the strong 1,3-diaxial interactions existing between the two O atoms and the

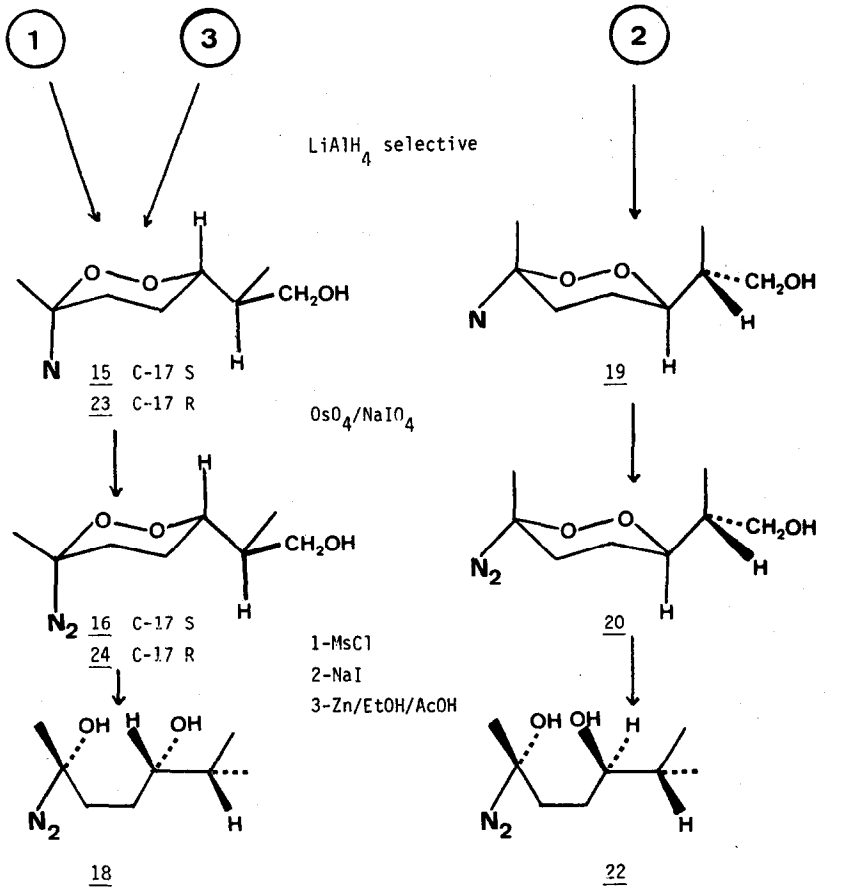

1
3
2
LiAlH4 selective
CH2OH
15 C-17 S
23 C-17 R
19
OsO4/NaIO4
16 C-17 S
24 C-17 R
20
1-MsCl
2-NaI
3-Zn/EtOH/AcOH
18
22

Fig. 2.

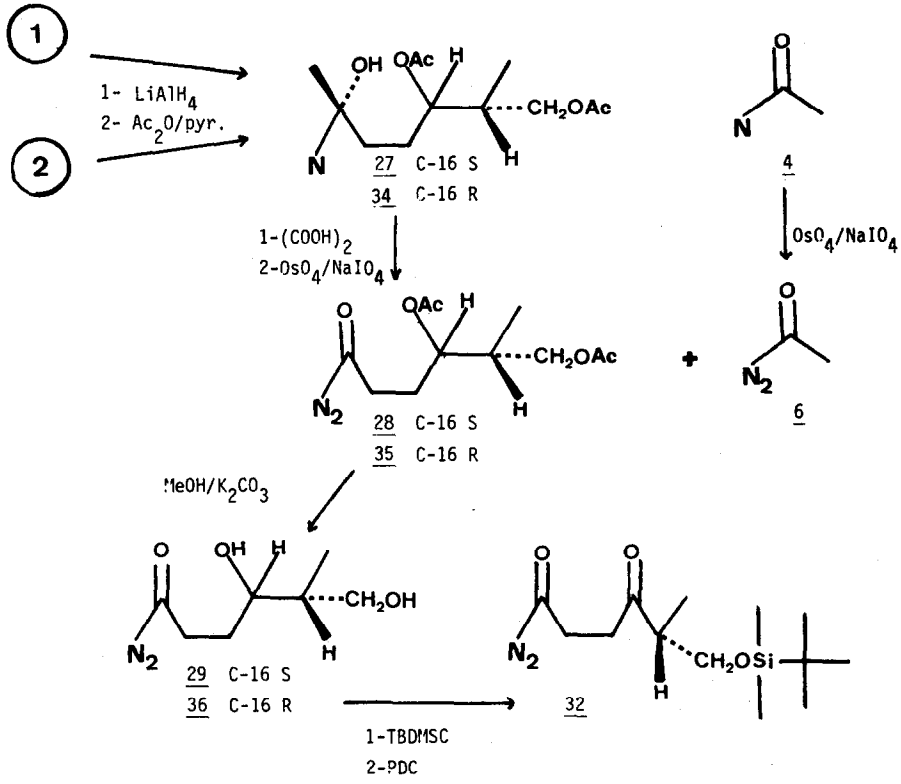
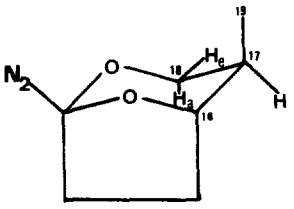
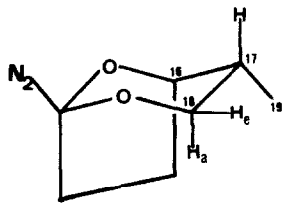

1
2
1- LiAlH4
2- Ac2O/pyr.
27 C-16 S
34 C-16 R
4
OsO4/NaIO4
1-(COOH)2
2-OsO4/NaIO4
28 C-16 S
35 C-16 R
6
MeOH/K2CO3
29 C-16 S
36 C-16 R
32
1-TBDMSC
2-PDC

Fig. 3.

Table 2. Characteristic chemical shifts and coupling constants of **30** and **37**.



30



37

H_{18e}	4.01 ppm	$J_{18e-18a}$	= 11 Hz	H_{18e}	3.75 ppm	$J_{18e-18a}$	= 11 Hz
H_{18a}	3.48 ppm	J_{18e-17}	= 3 Hz	H_{18a}	3.35 ppm	J_{18e-17}	= 5 Hz
H_{16}	4.17 ppm	J_{18a-17}	~ 1 Hz	H_{16}	4.22 ppm	J_{18a-17}	= 11 Hz
$19-CH_3$	1.25 ppm			$19-CH_3$	0.69 ppm		

$^{19}CH_3$ in an axial position. Such a deshielding is well known for axial versus equatorial C-5 protons and alkyl groups in 1,3-dioxanes.[46,47]

It was readily demonstrated that sigmosceptrellin-C methyl ester (**3**) is the C-17 epimer of sigmosceptrellin-A methyl ester (**1**) by transforming **3** into the ketodiol **18**, using the same sequence of reactions described above for the hydrogenolysis of the primary alcohol of **15** and **19** (Fig. 2).

We know from the X-ray diffraction analysis[43] that, in the solid state, the stable conformation of the 1,2-dioxane ring in **1** is a chair form having the $^{20}CH_3$ equatorial and the HC-16 axial. This conformation is probably also preferred in $CHCl_3$ solution since the coupling constants (deduced by computer simulation and decoupling experiments) for HC-16 ($\delta = 4.23$ ppm, $J_{16,17} = 7$ Hz, $J_{16,15a} = 7$ Hz and $J_{16,15e} = 4$ Hz) best agree with an axial orientation of the latter. A similar situation also prevails in **3** ($\delta = 4.15$ ppm, $J_{16,17} = 7$ Hz, $J_{16.15a} = 7$ Hz, $J_{16,15e} = 3$ Hz). In **2**, the coupling constants for HC-16 ($\delta = 4.14$ ppm, $J_{16,17} = 7$ Hz, $J_{16.15a} = 7$ Hz, $J_{16.15e} = 4$ Hz) indicate that this proton is also axial, suggesting that the stable conformation is the inverted chair form where the $^{20}CH_3$ is axial, the two other substituents being equatorial. This inversion of conformation is further supported by the variation of chemical shifts of the $^{20}CH_3$. Indeed in **1**, **3** and their derivatives (**5**, **15**, **16**, **17**, **23**, **24** and **25**) the singlet attributable to this Me group appears at about 1.05 ppm while it appears at about 1.25 ppm in **2** and its derivatives (**13**, **19**, **20** and **21**). This is reminiscent of the situation which prevails in 1,3-dioxanes (*vide supra*).

The absolute configuration of **2** has been established as follows: the ketone **13**, obtained by oxidative cleavage of the $\Delta^{4(24)}$-exomethylene function of **2**, exhibits a positive Cotton effect at 296 nm. Application of the octant rule requires the angular Me ($^{23}CH_3$) to be on a R C atom (C-5). Hence, sigmosceptrellin-B methyl ester has the same absolute configuration as ilimaquinone[48] and is antipodal with the closely related rearranged sesquiterpene hydroquinone avarol.[49] The absolute configuration of **2** is thus: 5R, 8R, 9S, 10R, 13S, 16R and 17S. Those of **1** (5R, 8R, 9S, 10R, 13S, 16S and 17S) and **3** (5R, 8R, 9S, 10R, 13S, 16S and 17R) follow from the already described chemical intercorrelations.

In conclusion, it follows from all these data that sigmosceptrellin-A, -B and-C, the three norsesterterpene peroxides from the ichthyotoxic fraction of the sponge *Sigmosceptrella laevis*, are represented by formulae **1a**, **2a** and **3a** respectively (including absolute configuration).

Although the carbon skeleton of the sigmosceptrellins has not been previously described, they are not the sole norsesterterpenes isolated from a Porifera. Indeed, recently Kashman[50] reported the isolation of muqubilin, a C-24 isoprenoid from *Prianos sp.* The bicyclic part of the sigmosceptrellins (rearranged drimane skeleton) is shared with other sponge metabolites (e.g. ilimaquinone,[48] avarol[49,25] and isospongiaquinone[51]). Moreover, although cyclic peroxides are quite rare in nature,[52] several ones including lipids,[30,34,53,54] steroids[55,56] and a norsesterterpene[50] have already been isolated from sponges.

EXPERIMENTAL

The following instruments were used for measuring the physical data: IR: Pye Unicam SP 1000; UV Pye Unicam SP 800; ^1H NMR: Perkin–Elmer R24B, Jeol JNM/MH 100 or Brucker HFX 270; ^{13}C NMR: Brucker WP 60; Rotation power: Perkin–Elmer 141; MS: Finnigan 3000 D, Micromass 7070 or AEI MS 902. The NMR spectra were recorded in $CDCl_3$ sol. Chemical shifts are quoted in δ values (ppm) downfield from TMS as internal standard. The tlc were performed on Polygram Sil G pre-coated plates (Macherey-Nagel). All described compounds were homogeneous in tlc. Column chromatographies were performed on Macherey-Nagel Kieselgel-60 (0.063–0.2 mm).

Isolation of compounds **1**, **2**, **3** *and* **4**

Sun-dried specimens (200 g) of the sponge *Sigmosceptrella laevis* were extracted with CH_2Cl_2 and MeOH. The CH_2Cl_2 extract was evaporated under reduced pressure to give a viscous oil (17.8 g – 8.9% dry wt), which was chromatographed on SiO_2 column (eluent: hexane/AcOEt 9:1→4:6). This afforded 1.2 g of a mixture of sigmosceptrellin-A and -B methyl esters (**1**, **2**) and the methylketone **4**, as well as a mixture of sigmosceptrellin-A, -B and -C which, on methylation with CH_2N_2, yielded 2.6 g of **1**,

2, **3**. The latter were separated by several chromatographies on SiO_2 ("Lobar" column, eluent: hexane/CH_2Cl_2 3:7) leading to 1.4 g of **2**, 0.6 g of **1** and 0.2 g of **3**.

Compound **1**: $C_{25}H_{42}O_4$ (by HRMS: calculated 406.3083, observed 406.3101); ^1H NMR: see Table 1; ^{13}C NMR: 174.2 s, 160.5 s, 102.6 t, 81.1 d, 80.2 s, 51.8 q, 48.7 d, 42.6 d, 40.1 s, 39.1 s, 37.5 t, 36.7 t, 33.1 t, 32.5 t, 31.3 t, 28.7 t, 27.5 t, 27.3 t, 24.0 t, 22.5 q, 21.8 t, 20.8 q, 18.3 q, 15.9 q, 12.4 q; MS: 406 (M^+, 0.1), 391 ($M^+ - 15$, 0.3), 375 ($M^+ - 31$, 1.5), 301 (2), 203 (0.5), 192 (10), 191 (54), 190 (14), 189 (18), 177 (10), 175 (15), 171 (19), 135 (31), 133 (10), 123 (12), 122 (14), 121 (32), 120 (12), 119 (15), 109 (37), 107 (19), 99 (16), 95 (100), 93 (33), 91 (14); IR(film); 1750, 1640 and 890 cm^{-1}; $[\alpha]_D = +53.4°$ ($CHCl_3$, $c = 0.618$).

Compound **2**. ^1H NMR: see Table 1; ^{13}C NMR: 174.3 s, 160.5 s, 102.6 t, 81.3 d, 80.3 s, 51.8 q, 48.5 d, 42.9 d, 40.0 s, 39.0 s, 37.4 t, 36.5 d, 33.0 t, 32.5 t, 31.9 t, 30.7 t, 28.6 t, 27.5 t, 23.5 t, 21.7 t, 20.9 q, 20.8 q, 18.3 q, 15.9 q, 13.5 q; MS: 406 (M^+, 0.1), 391 ($M^+ - 15$, 0.2), 375 ($M^+ - 31$, 0.5), 301 (1.5), 203 (2), 192 (11), 191 (58), 190 (16), 189 (10), 175 (10), 135 (30), 123 (12), 122 (12), 121 (32), 119 (10), 109 (34), 107 (26), 99 (15), 95 (100), 93 (29), 91 (13); IR (film): 1750, 1640 and 890 cm^{-1}; $[\alpha]_D = -61.2°$ ($CHCl_3$, $c = 0.446$).

Compound **3**. ^1H NMR: see Table 1; ^{13}C NMR: 174.2 s, 160.7 s, 102.6 t, 80.9 d, 80.2 s, 51.8 q, 48.8 d, 43.0 d, 40.2 s, 39.2 s, 37.5 t, 36.7 t, 33.1 t, 32.3 t, 31.2 t, 28.7 t, 27.9 t, 27.6 t, 23.7 t, 23.5 q, 21.9 t, 20.9 q, 18.4 q, 15.9 q, 13.2 q; MS: 406 (M^+, 0.1), 391 ($M^+ - 15$, 3), 375 ($M^+ - 31$, 2), 301 (4), 203 (4), 191 (57), 190 (14), 189 (10), 177 (10), 175 (10), 135 (26), 122 (11), 121 (28), 109 (30), 107 (20), 99 (15), 95 (100), 93 (21); IR(film): 1750, 1640 and 890 cm^{-1}; $[\alpha]_D = +42.2°$ ($CHCl_3$, $c = 9.67$).

Methyl ketone **4** could not be separated by SiO_2 column chromatography from **1** and **2**. Accordingly the mixture was submitted to $NaBH_4$ reduction followed by SiO_2 chromatography affording alcohol **4a**. The original ketone **4** was recovered by Jones oxidation of **4a**.

Compound **4**. M^+ 262, $C_{18}H_{30}O$; IR (film): 1725, 1640 and 890 cm^{-1}; ^1H NMR (60 MHz): 0.78 (s, 3H), 0.80 (d, 3H, J = 5), 1.05 (s, 3H), 2.13 (s, 3H), 4.51 (bs, 2H); $[\alpha]_{579} = -41.1°$ ($CHCl_3$, $c = 1.24$).

Compound **4a**: M^+ 264, $C_{18}H_{32}O$; IR (film): 3380, 1640 and 890 cm^{-1}; ^1H NMR (60 MHz): 0.73 (s, 3H), 0.77 (d, 3H, J = 5), 1.02 (s, 3H), 1.15 (d, 3H, J = 6), 3.69 (m, 1H), 4.51 (bs, 2H).

Oxidative cleavage of **4**

A soln of **4** (38 mg) and OsO_4 (50 mg) in anhydrs pyridine was stirred at room temp for 14 h. Treatment of the mixture with $NaHSO_3$ for 1 hr, followed by extraction with CH_2Cl_2 and evaporation of the solvent yielded 35 mg of a gum which was immediately dissolved in MeOH/H_2O 9:1 (5 ml). An excess of $NaIO_4$ was added and the mixture was stirred for 15 hr. After extraction and purification by SiO_2 column chromatography, **6** (29 mg) was obtained.

Compound **6**. M^+ 264, $C_{17}H_{28}O_2$; (IR film): 1725 and 1715 cm^{-1}; ^1H NMR (60 MHz): 0.78 (d, 3H, J = 5), 0.80 (s, 3H), 1.13 (s, 3H), 2.13 (s, 3H); $[\alpha]_{579} = -19.8°$ ($CHCl_3$, $c = 2.825$).

Catalytic hydrogenation of **1**

A soln of **1** (49 mg) in EtOH containing 10% Pd/C (5 mg) was stirred under an atmosphere of H_2 for 4 hr. The catalyst was removed by filtration and diol **7** (48 mg) was obtained by SiO_2 column chromatography.

Compound **7**. $C_{25}H_{46}O_4$, no molecular ion, $M^+ - 17$, 393; IR (KBr): 3300, 1750 and 1250 cm^{-1}; ^1H NMR (60 MHz): 0.68 (s, 3H), 0.73 (d, 3H, J = 5), 0.75 (s, 3H), 0.75 (d, 3H, J = 5), 1.15 (s, 3H), 1.15 (d, 3H, J = 7), 2.55 (quintet, 1H, J = 7), 3.60 (m, 1H), 3.67 (s, 3H).

Catalytic hydrogenation of **2**

Compound **2** (95 mg) was catalytically reduced to **10** (90 mg) as described for **1**

Compound **10**. $C_{25}H_{46}O_4$, no molecular ion, $M^+ - 17$, 393; IR(KBr): 3400, 1750 and 1250 cm^{-1}; ^1H NMR (60 MHz): 0.70 (d, 3H, J = 5), 0.70 (s, 3H), 0.73 (d, 3H, J = 5), 0.75 (s, 3H), 1.15 (s, 3H), 1.18 (d, 3H, J = 7), 2.45 (m, 1H), 3.68 (s, 3H), 3.80 (m, 1H).

Jones oxidation of **7** *and* **10**

Compound **7** (50 mg) was dissolved in acetone and Jones reagent was added dropwise until an orange coloration was maintained. The mixture was stirred at room temp for 10 mn Extraction with CH_2Cl_2 and chromatography on SiO_2 yielded **8** (35 mg). The same product is obtained from **10** using the same experimental procedure.

Compound **8**. $C_{25}H_{42}O_3$, M^+ 390; IR (film): 1720 and 1640 cm^{-1}; UV (MeOH) λ_{max} 250 nm ($\epsilon = 10000$); ^1H NMR (100 MHz): 0.70 (s, 3H), 0.75 (d, 6H, J = 5), 0.76 (s, 3H), 1.29 (s, 3H), 1.79 (dd, 3H), 3.14 (t, 2H), 3.68 (s, 3H); ^{13}C NMR ($CDCl_3$/TMS): 170.4 s, 170.1 s, 96.7 s, 89.1 s, 50.8 d, 49.8 t, 46.1 q, 39.4, 38.5, 37.2, 36.5, 35.1, 33.4, 32.3, 31.4, 30.9, 27.4, 24.9, 21.6, 18.5, 16.0, 15.1, 13.2, 11.3.

PCC oxidation of **10** *and* **7**

A solon of **10** (30 mg) and pyridinium chlorochromate (PCC) (10 mg) in anhydrs CH_2Cl_2 was stirred for 5 hr at room temp. Extraction and chromatography on SiO_2 afforded 18.9 mg of **9**. The same product is obtained from **7**, under the same reaction conditions.

Compound **9**. M^+ 320, $C_{21}H_{36}O_2$; IR (film): 1780 cm^{-1}; ^1H NMR (100 MHz): 0.69 (s, 3H), 0.72 (d, 3H, J = 5), 0.80 (d, 3H, J = 5), 0.80 (s, 3H), 1.36 (s, 3H), 2.52 (d, 1H), 2.62 (dd, 1H).

Oxidative cleavage of **1**

Oxidative cleavage of the $\Delta^{4(24)}$-double bond of **1** was carried out as described for **4**. 50 mg of **1** afforded 48 mg of **5**.

Compound **5**. M^+ 408, $C_{24}H_{40}O_5$; IR (film): 1750, 1715 and 1250 cm^{-1}; ^1H NMR (100 MHz): 0.79 (d, 3H, J = 5), 0.80 (s, 3H), 1.05 (s, 3H), 1.10 (d, 3H, J = 7), 1.11 (s, 3H), 2.11 (m, 1H), 2.26 (m, 1H), 2.56 (quintet, 1H, J = 7), 3.67 (s, 3H, 4.25 (m, 1H).

Catalytic hydrogenation of **5**

Catalytic hydrogenation of **5** (30 mg) for 20 hr afforded, after chromatography on SiO_2, **11** (26 mg).

Compound **11**. $C_{24}H_{42}O_5$, no molecular ion, $M^+ - 18$:392; IR (film): 3460, 3380, 1750, 1715 and 1250 cm^{-1}; ^1H NMR (60 MHz): 0.79 (d, 3H, J = 5), 0.80 (s, 3H), 1.15 (s, 3H), 1.16 (s, 3H), 1.22 (d, 3H, J = 7), 3.60 (m, 1H), 3.67 (s, 3H).

Oxidative cleavage of **2**

Oxidative cleavage of the $\Delta^{4(24)}$ double bond of **2** (40 mg) was performed as described for **4**. This yielded 28 mg of ketone **13**.

Compound **13**. M^+ 408, $C_{24}H_{40}O_5$; IR (film): 1750, 1715 and 1250 cm^{-1}; ^1H NMR (100 MHz): 0.78 (d, 3H, J = 5), 0.80 (s, 3H), 1.11 (s, 3H), 1.22 (d, 3H, J = 7), 1.23 (s, 3H), 2.09 (m, 1H, 2.25 (m, 1H), 2.65 (quintet, 1H, J = 7), 3.68 (s, 3H, 4.11 (m, 1H).

Catalytic hydrogenation of **13**

Compound **13** (28 mg) hydrogenated as previously described for **1** and **2**, afforded diol **14** (24 mg).

Compound **14**. $C_{24}H_{42}O_5$, no molecular ion, $M^+ - 18$:392; IR (KBr): 3360, 1750, 1715 and 1250 cm^{-1}; ^1H NMR (60 MHz): 0.79 (d, 3H, J = 5), 0.81 (s, 3H), 1.14 (s, 3H), 1.15 (s, 3H), 1.20 (d, 3H, J = 7), 3.67 (s, 3H), 3.80 (m, 1H).

Jones oxidation of **14** *and* **11**

Compound **14** (24 mg) dissolved in acetone was treated with Jones reagent for 15 mn. Compound **12** (18 mg) was obtained after usual work-up and chromatography on SiO_2. The same compound **12** was obtained on Jones oxidation of **11**.

Compound **12**. M^+ 390, $C_{24}H_{38}O_4$; IR (film): 1715 and 1640 cm^{-1}; ^1H NMR (270 MHz): 0.78 (d, 3H, J = 5), 0.82 (s, 3H), 1.14(s, 3H), 1.28 (d, 3H, J = 7), 1.29(s, 3H), 1.79 (bs, 3H), 2.08 (m, 1H), 2.21 (bdd, 1H), 2.58 (d, 1H, J = 7), 3.14 (m, 2H), 3.71 (s, 3H); $[\alpha]_{579} = +7.3°$ ($CHCl_3$, c = 1.95) from **11** and $[\alpha]_{579} = +7.2°$ ($CHCl_3$, $c = 1.60$) from **14**.

$LiAlH_4$ *selective reduction of* **1**

A soln of **1** (121 mg) in dry THF (10 ml) containing $LiAlH_4$ (27 mg) was stirred for 3.30 h at room temp. Addition of a satd $MgSO_4$ soln and extraction with CH_2Cl_2 yielded 110 mg of an oil,

which was chromatographed on SiO_2 (eluent: hexane/acetone 8:2) to give 70 mg of **15**.

Compound **15**. M^+ 378, $C_{24}H_{42}O_3$; IR (film): 3400, 1640 and 890 cm^{-1}; ^1H NMR (60 MHz): 0.75 (s, 3H), 0.80 (d, 3H, J = 5), 0.91 (d, 3H, J = 7), 1.05 (s, 6H), 3.58 (AB, 2H), 3.93 (m, 1H), 4.50 (bs, 2H).

$LiAlH_4$ *selective reduction of* **2**

Under the same reaction conditions as for **1**, 185 mg of **2** yielded 135 mg of **19**.

Compound **19**. M^+ 378, $C_{24}H_{42}O_3$; IR (film): 3450, 1640 and 890 cm^{-1}; ^1H NMR (60 MHz): 0.73 (s, 3H), 0.78 (d, 3H, J = 5), 0.95 (d, 3H, J = 7), 1.03 (s, 3H) 1.27 (s, 3H), 3.60 (AB, 2H), 4.06 (m, 1H), 4.48 (bs, 2H).

$LiAlH_4$ *selective reduction of* **3**

Under the same reaction conditions as for **1** and **2**, 45 mg of **3** afforded 38 mg of **23**.

Compound **23**. M^+ 378, $C_{24}H_{42}O_3$; IR (film): 3400, 1640 and 890 cm^{-1}; ^1H NMR (60 MHz): 0.75 (s, 3H), 0.82 (d, 3H, J = 5), 0.95 (d, 3H, J = 7), 1.05 (s, 6H), 3.58 (AB, 2H), 4.13 (m, 1H), 4.48 (bs, 2H).

Cleavage of **15**

Treatment of **15** (70 mg) with OsO_4 (100 mg, 20 h) and $NaIO_4$ (100 mg, 5 hr) as described previously, afforded compound **16** (60 mg).

Compound **16**. M^+ 380, $C_{23}H_{40}O_4$; IR (film): 3450 and 1720 cm^{-1}; ^1H NMR (60 MHz): 0.81 (d, 3H, J = 5), 0.83 (s, 3H), 0.91 (d, 3H, J = 7), 1.05 (s, 3H), 1.15 (s, 3H), 3.60 (AB, 2H), 3.96 (m, 1H).

Cleavage of **19**

Treatment of **19** (130 mg) with OsO_4 (120 mg, 20 hr) and $NaIO_4$ (100 mg, 20 hr) as described before, yielded 106 mg of **20**).

Compound **20**. M^+ 380, $C_{23}H_{40}O_4$; IR (film): 3500 and 1720 cm^{-1}; ^1H NMR (60 MHz): 0.80 (s, 3H), 0.85 (d, 3H, J = 5), 0.96 (d, 3H, J = 7), 1.10 (s, 3H), 1.28 (s, 3H), 3.60 (AB, 2H), 4.05 (m, 1H).

Cleavage of **23**

Treatment of **23** (38 mg) with OsO_4 (100 mg, 70 h) and $NaIO_4$ (50 mg, 20 hr) yielded 33 mg of **24**.

Compound **24**: M^+ 380, $C_{23}H_{40}O_4$; IR (film): 3500 and 1720 cm^{-1}; ^1H NMR (60 MHz): 0.83 (s, 3H), 0.84 (d, 3H, J = 5), 0.95 (d, 3H, J = 7), 1.05 (s, 3H), 1.13 (s, 3H), 3.60 (AB, 2H), 4.10 (m, 1H).

Mesylation of **16**

A soln of **16** (60 mg) in dry pyridine (10 ml) and mesylchloride (0.13 ml) was stirred for a 3 hr 1/2. Excess reagent was destroyed by addition of water. Extraction with CH_2Cl_2 and chromatography on SiO_2 (hexane/acetone 7:3) gave 70 mg of **17**.

Compound **17**. M^+ 458, $C_{24}H_{42}O_6S$; IR (film): 1720 and 1180 cm^{-1}; ^1H NMR (60 MHz): 0.80 (s, 3H), 0.83 (d, 3H, J = 5), 1.00 (d, 3H, J = 7), 1.03 (s, 3H), 1.10 (s, 3H), 2.95 (s, 3H), 3.95 (m, 1H), 4.15 (AB, 2H).

Mesylation of **20**

Treatment of **20** (105 mg) with mesylchloride (0.23 ml) as above, gave 110 mg of **21**

Compound **21**. M^+ 458, $C_{24}H_{42}O_6S$; IR (film): 1720 and 1180 cm^{-1}; ^1H NMR (60 MHz): 0.80 (s, 3H), 0.81 (d, 3H, J = 5), 1.03 (d, 3H, J = 7), 1.11 (s, 3H), 1.28 (s, 3H), 3.00 (s, 3H), 4.05 (m, 1H), 4.16 (AB, 2H).

Mesylation of **24**

Treatment of **24** (33 mg) with mesylchloride (0.07 ml) yielded 35 mg of **25**.

Compound **25**. M^+ 458, $C_{24}H_{42}O_6S$; IR (film): 1715 and 1180 cm^{-1}; ^1H NMR (60 MHz): 0.81 (s, 3H), 0.82 (d, 3H, J = 5), 0.98 (d, 3H, J = 7), 1.03 (s, 3H), 1.13 (s, 3H), 2.99 (s, 3H), 4.05 (m, 1H), 4.11 (AB, 2H).

NaI/Zn *reduction of* **17**

A soln of **17** (70 mg) and NaI (38 mg) in acetone was refluxed for 14 hr. After chromatography on SiO_2, 58 mg of the corresponding iodide were obtained. A soln of this iodide in EtOH was refluxed with Zn/AcOH for 2 hr, yielding 38 mg of **18**.

Compound **18**. M^+ 366, $C_{23}H_{42}O_3$; IR (film): 3350 and 1720 cm^{-1}; ^1H NMR (100 MHz): 0.77 (d, 3H, J = 5), 0.80 (s, 3H), 0.91 (d, 6H, J = 7), 1.11 (s, 3H), 1.14 (s, 3H), 3.32 (m, 1H); $[\alpha]_{579} = -1.5°$ ($CHCl_3$, c = 3.71).

NaI/Zn *reduction of* **21**

Treatment of **21** (60 mg) under the same conditions as **17**, afforded 40 mg of **22**.

Compound **22**. M^+ 366, $C_{23}H_{42}O_3$; IR (film): 3300 and 1720 cm^{-1}; ^1H NMR (100 MHz): 0.77 (d, 3H, J = 5), 0.80 (s, 3H), 0.91 (d, 6H, J = 7), 1.12 (s, 3H), 1.14 (s, 3H), 3.31 (m, 1H); $[\alpha]_{579} = -22.3°$ ($CHCl_3$, c = 1.72).

NaI/Zn *reduction of* **25**

Treatment of **25** (35 mg) under the same conditions as **17**, yielded 21 mg of **18**. $[\alpha]_{579} = -1.7°$ ($CHCl_3$, c = 1.0); IR and ^1H NMR (100 MHz) identical to those of the derivative originating from **17**.

$LiAlH_4$ *reduction of* **1**

$LiAlH_4$ (700 mg) was added to a soln of **1** (770 mg) in dry THF (50 ml). The soln was stirred at room temp for 7 hr. Excess reagent was destroyed by addition of a satd $MgSO_4$ soln. The mixture was successively extracted with CH_2Cl_2 and $CHCl_3$–EtOH 3:2, affording 709 mg of a gum which was purified by chromatography on SiO_2 (eluent: hexane/acetone 7:3), to give 540 mg of triol **26**.

Compound **26**: $C_{24}H_{44}O_3$, no molecular ion, $M^+ - 17$:363; IR (film): 3400, 1640 and 890 cm^{-1}; ^1H NMR (60 MHz): 0.75 (s, 3H), 0.78 (d, 3H, J = 5), 0.85 (d, 3H, J = 7), 1.05 (s, 3H), 1.15 (s, 3H), 3.47 (m, 1H), 3.65 (AB, 2H), 4.55 (bs, 2H).

$LiAlH_4$ *reduction of* **2**

$LiAlH_4$ (1 g) was added to a soln of **2** (1.2 g) in dry THF (50 ml). The solution was stirred at room temp for 72 hr. Usual work up and purification afforded 640 mg of triol **33**.

Compound **33**. $C_{24}H_{44}O_3$, no molecular ion, $M^+ - 17$; 363; IR (film): 3400, 1640 and 890 cm^{-1}; ^1H NMR (60 MHz): 0.77 (s, 3H), 0.83 (d, 3H, J = 5), 0.91 (d, 3H, J = 7), 1.04 (s, 3H), 1.16 (s, 3H), 3.60 (m, 1H), 3.67 (AB, 2H), 4.48 (bs, 2H).

Acetylation of **26**

A soln of **26** (540 mg) and Ac_2O (1 ml) in dry pyridine (20 ml) was stirred at room temp for 72 hr. After extraction and chromatography 630 mg of **27** were obtained.

Compound **27**. M^+ 464, $C_{28}H_{48}O_5$; IR (film): 3550, 1750, 1640, 1240 and 890 cm^{-1}; ^1H NMR (60 MHz): 0.75 (s, 3H), 0.80 (d, 3H, J = 5), 0.98 (d, 3H, J = 7), 1.05 (s, 3H), 1.13 (s, 3H), 2.07 (s, 6H), 4.03 (AB, 2H), 4.55 (bs, 2H), 4.93 (m, 1H).

Acetylation of **33**

A soln of triol **33** (570 mg) and Ac_2O (1.5 ml) in dry pyridine (20 ml) was stirred at room temp for 20 hr. After extraction and chromatography, 670 mg of **34** were obtained.

Compound **34**. M^+ 464, $C_{28}H_{48}O_5$; IR (film): 3500, 1750, 1640, 1245 and 890 cm^{-1}; ^1H NMR (60 MHz): 0.74 (s, 3H), 0.78 (d, 3H, J = 5), 0.95 (d, 3H, J = 7), 1.04 (s, 3H), 1.15 (s, 3H), 2.05 (s, 6H), 3.98 (m, 2H), 4.53 (bs, 2H), 4.98 (m, 1H).

Dehydration of **27**

Oxalic acid (120 mg) was added to a soln of **27** (630 mg) in benzene. The mixture was refluxed for 7 hr. Usual work-up and chromatography on SiO_2 (hexane/acetone 9:1) yielded 520 mg of a mixture of double bond isomers. 480 mg of this mixture were hydroxylated with OsO_4 (700 mg, one week) and the resulting crude mixture was treated with $NaIO_4$ for 65 hr. Purification by SiO_2 chromatography yielded 50 mg of **28** and 75 mg of methylketone **6**.

Compound **28**. M^+ 450, $C_{26}H_{42}O_6$; IR (film): 1750, 1720 and

1250 cm^{-1}; ^1H NMR (60 MHz): 0.83 (s, 3H), 0.83 (d, 3H, J = 5), 0.95 (d, 3H, J = 7), 1.13 (s, 3H), 2.05 (s, 6H), 4.00 (AB, 2H), 4.85 (m, 1H).

Compound **6**. $[\alpha]_{579} = -20.9°$ ($CHCl_3$, c = 4.77); IR and ^1H NMR (60 MHz) identical to those of the derivative originating from **4**.

Dehydration of **34**

The same treatment applied to **34** (670 mg) afforded 58 mg of **35** and 68 mg of methylketone **6**.

Compound **35**. M^+ 450, $C_{26}H_{42}O_6$; IR (film): 1750, 1720 and 1250 cm^{-1}; ^1H NMR (60 MHz): 0.83 (s, 3H), 0.84 (d, 3H, J = 5), 0.95 (d, 3H, J = 7), 1.13 (s, 3H), 2.03 (s, 6H), 3.93 (AB, 2H), 4.90 (m, 1H).

Compound **6**. $[\alpha]_{579} = -20.1°$ ($CHCl_3$, c = 6.06); IR and ^1H NMR (60 MHz) identical to those of the derivative originating from **4**.

Deacetylation and silylation of **28**.

28 (25 mg) was stirred overnight with 5 ml of satd K_2CO_3/MeOH. Extraction with CH_2Cl_2 afforded a gum which, after chromatography, yielded the ketal **30**. In another experiment, the chromatography was omitted and the crude diol (16 mg) was immediately treated with 40 mg of TBDMCS, 6 mg of DMAP, 0.1 ml of NEt_3 in CH_2Cl_2. The soln was stirred for 96 hr. Extraction and chromatography (hexane/acetone 8:2) yielded 18 mg of the TBDMS ether **31**.

Compound **30**. M^+ 348, $C_{22}H_{36}O_3$; IR (film): 1720 cm^{-1}; ^1H NMR (270 MHz): 0.78 (d, 3H, J = 5), 0.79 (s, 3H), 1.09 (s, 3H), 1.25 (d, 3H, J = 2), 2.1 (bdd, 1H), 2.55 (sextet, 1H), 3.48 (bd, 1H), 4.01 (dd, 1H), 4.17 (bd, 1H).

Compound **31**. $C_{28}H_{52}O_4Si$, no molecular ion, $M^+ - 57$:423; IR (film): 3550, 1720, 1100, 840 and 780 cm^{-1}.

Deacetylation and silylation of **35**

Deacetylation of **35** (20 mg) followed by SiO_2 chromatography as described for **28** afforded ketal **37**. Likewise, deacetylation immediately followed by silylation yielded compound **38**.

Compound **37**. M^+ 348, $C_{22}H_{36}O_3$; IR (film): 1720 cm^{-1}; ^1H NMR (270 MHz): 0.69 (d, 3H, J = 7), 0.79 (d, 3H, J = 5), 0.80 (s, 3H), 1.11 (s, 3H), 2.17 (bdd, 1H) 2.55 (sextet, 1H), 3.35 (dd, 1H), 3.75 (dd, 1H), 4.22 (bs, 1H).

Compound **38**. $C_{28}H_{52}O_4Si$ no molecular ion, M^+ −57:423; IR (film): 3500, 1720, 1100, 845 and 780 cm^{-1}.

Oxidation of **31**

PDC (20 mg) was added to a soln of **31** (12 mg) in 2 ml of anhyd CH_2Cl_2. The soln was stirred at room temp for 12 hr. The solid residue was removed by filtration and the solvent evaporated. Chromatography on SiO_2 (hexane/ether 6:4) yielded 5 mg of **32**.

Compound **32**. $C_{28}H_{50}O_4Si$, no molecular ion, $M^+ - 57$:421; IR (film): 1720, 1100, 840 and 780 cm^{-1}; ^1H NMR (270 MHz): 0.02 (s, 3H), 0.025 (s, 3H), 0.80 (d, 3H, J = 5), 0.83 (s, 3H), 0.86 (s, 9H), 1.05 (d, 3H, J = 7), 1.13 (s, 3H), 3.70 (AB from ABX system, 2H).

Oxidation of **38**

PDC oxidation of **38** (10 mg) as described for **31**, yielded 2 mg of **32**.

Lethal dose (LD) determination

The adequate amounts of the extracts or fractions to be tested are each dissolved in 1 ml of EtOH. The resulting solns are poured into beakers (250 ml) containing 100 ml of tap water. The lethal dose is determined by immersion of samples of 3 fishes (*Lebistes reticulatus*) in the different concentrations of the toxin (generally 50 to 1 mg/l). For each concentration the number of dead fishes after 24 hr is noted.

The LD of the CH_2Cl_2 extract of *Sigmosceptrella laevis* was found to be 25 mg/l while that of the mixture of acids **1a**, **2a** and **3a** was 5 mg/l.

Acknowledgements—We thank Mr. J. Pierret for his participation in the collection of the animal, Dr. P. A. Thomas for its identification and Dr. R. Ottinger for the ^{13}C NMR spectra. One of us (M.A.) is indebted to the "Institut pour l'Encouragement de la Recherche Scientifique dans l'Industrie et l'Agriculture" (IRSIA) for financial support.

REFERENCES

[1]Part XLIV: N. Capelle, J. C. Braekman, D. Daloze and B. Tursch *Bull. Soc. Chim. Belg.* **89**, 399 (1980).
[2]King Leopold III Biological Station, Laing Island, Papua-New Guinea Contribution N° 28.
[3]Maître de Recherches du F.N.R.S.
[4]G. J. Bakus and G. Green, *Science*, **185**, 951 (1974).
[5]G. Green, *Mar. Biol.* **40**, 207 (1977).
[6]G. J. Bakus, *Science* **211**, 497 (1981).
[7]D. J. Faulkner, Communication presented at the 3*rd Int. Symp. Marine Natural Products*, Brussels, September 1980.
[8]J. E. Randall and W. D. Hartman, *Mar. Biol.* **1**, 216 (1968).
[9]L. Minale, *Pure and Applied Chem.* **48**, 7 (1976).
[10]L. Minale, G. Cimino, S. De Stefano and G. Sodano *Fortschr. Chem. Org. Naturst.* **33**, 1 (1976).
[11]F. J. Schmitz, K. H. Hollenbeak and D. C. Campbell, *J. Org. Chem.* **43**, 3916 (1978).
[12]R. P. Walker, J. E. Thompson and D. J. Faulkner, *Ibid*, **45**, 4976 (1980).
[13]B. J. Burreson, P. J. Scheuer, J. Finer and J. Clardy, *J. Am. Chem. Soc.* **97**, 4673 (1975).
[14]M. R. Hagadone, B. J. Burreson, P. J. Scheuer, J. Finer and J. Clardy, *Helv. Chim. Acta* **62**, 2484 (1979).
[15]G. Schulte, P. J. Scheuer, O. J. McConnell, *Ibid.* **63**, 2159 (1980).
[16]C. Charles, J. C. Braekman, D. Daloze, B. Tursch and R. Karlsson *Tetrahedron Letters* 1519 (1978).
[17]Y. Kashman, A. Groweiss and U. Schmueli, *Ibid*, **21**, 3629 (1980).
[18]I. Neeman, L. Fishelson and Y. Kashman, *Mar. Biol.* **30**, 293 (1975).
[19]For leading references see W. Fenical, H. L. Sleeper, V. J. Paul, M. O. Stallard and H. H. Sun, *Pure and Appl. Chem.* **51**, 1865 (1979).
[20]P. R. Burkholder, *The ecology of marine antibiotics and coral reefs*, in *Biology and Geology of Coral Reefs* (Edited by R. O. Endean)., Vol. II, p. 117. Biology 1, Academic Press, (1973).
[21]R. M. Pfister and P. R. Burkholder, *J. Bacteriol.* **90**, 863 (1965).
[22]K. L. Rinehart, P. D. Shaw, L. S. Shield, J. B. Gloer, G. C. Harbour, M. E. S. Koker, D. Samain, R. E. Schwartz, A. A. Tymiak, D. L. Weller, G. T. Carter, M. H. G. Munro, R. G. Hughes, H. E. Renis, E. B. Swynenberg, D. A. Stringfellow, J. J. Vavra, J. H. Coats, G. E. Zurenko, S. L. Kuentzel, L. H. Li, G. J. Bakus, R. C. Brusca, L. L. Craft, D. N. Young J. L. Connor, *Pure and Appl. Chem.* **53**, 795 (1981).
[23]B. N. Ravi, H. P. Perzanowski, R. A. Ross, T. R. Erdman, P. J. Scheuer, J. Finer and J. Clardy, *Ibid.*, **51**, 1893 (1979).
[24]L. Minale, R. Riccio and G. Sodano, *Tetrahedron* **30**, 1341 (1974).
[25]P. Djura, D. B. Stierle, B. Sullivan, D. J. Faulkner, E. Arnold and J. Clardy, *J. Org. Chem.* **45**, 1435 (1980).
[26]S. J. Wratten and J. Meinwald, *Experientia* **37**, 13 (1981).
[27]G. T. Carter and K. L. Rinehart, *J. Am. Chem. Soc.* **100**, 4302 (1978).
[28]E. D. De Silva and P. J. Scheuer, *Tetrahedron Letters* 1611 (1980).
[29]D. E. McIntyre, D. J. Faulkner, D. Van Engen and J. Clardy, *Ibid.*, 4163 (1979).
[30]M. D. Higgs and D. J. Faulkner, *J. Org. Chem.* **43**, 3454 (1978).
[31]S. J. Wratten and D. J. Faulkner, *Tetrahedron Letters* 961 (1978).
[32]B. Sullivan, P. Djura, D. E. McIntyre and D. J. Faulkner, *Tetrahedron* **37**, 979 (1981).
[33]N. Capelle, J. C. Braekman, D. Daloze and B. Tursch, *Bull. Soc. Chim. Belg.* **89**, 399 (1980).
[34]D. B. Stierle and D. J. Faulkner, *J. Org. Chem.* **44**, 964 (1979).
[35]S. M. Al-Ogily and E. W. Knight-Jones, *Nature*, **265**, 728 (1977).
[36]D. Stocker, *Biol. Bull.* **155**, 615 (1978).
[37]M. Sara and J. Vacelet, *Ecologie des Démosponges* in *Traité*

de Zoologie, Anatomie, Systématique, Biologie: Spongiaires. (Edited by P. P. Grassé), p. 462–576. Masson, Paris (1973).
[38]J. Vacelet, *J. Microscopie Biol. Cell.* **23**, 271 (1975).
[39]F. J. Schmitz, R. S. Prasad and P. Schmidt, *J. Am. Chem. Soc.* **103**, 2467 (1981).
[40]K. Tachibana, P. J. Scheuer, Y. Tsukitani, H. Kikuchi, D. van Engen, J. Clardy, Y. Gopichand and F. J. Schmitz, *Ibid.* **103**, 2469 (1981).
[41]R. Kazlauskas, R. O. Lidgard and R. J. Wells, *Tetrahedron Letters* 3183 (1977).
[42]B. Tursch, Unpublished data.
[43]M. Albericci, M. Collart-Lempereur, J. C. Braekman, D. Daloze, B. Tursch, J. P. Declercq, G. Germain and M. van Meerssche, *Tetrahedron Letters* 2687 (1979).
[44]E. J. Corey and A. Venkateswarlu, *J. Am. Chem. Soc.* **94**, 6190 (1972).
[45]E. J. Corey and G. Schmidt, *Tetrahedron Letters* 399 (1979).
[46]E. L. Eliel and M. C. Knoeber, *J. Am. Chem. Soc.* **90**, 3444 (1968).
[47]J. Delmau and J. Duplan, *Tetrahedron Letters*, 2693 (1966).
[48]R. T. Luibrand, T. R. Erdman, J. J. Vollmer, P. J. Scheuer, J. Finer and J. Clardy, *Tetrahedron* **35**, 609 (1979).
[49]S. de Rosa, L. Minale, R. Riccio and G. Sodano, *J. Chem. Soc.* Perkin 1, 1408 (1976).
[50]Y. Kashman and R. Rotem, *Tetrahedron Letters* 1707 (1979).
[51]R. Kazlauskas, P. T. Murphy, R. G. Warden, R. J. Wells and J. F. Blount, *Austr. J. Chem.* **31**, 2685 (1978).
[52]W. Adam and A. J. Bloodworth, *Ann. Rep. Progr. Chem.* **75B**, 342 (1978).
[53]D. B. Stierle and D. J. Faulkner, *J. Org. Chem.* **45**, 3396 (1980).
[54]R. J. Wells, *Tetrahedron Letters* 2637 (1976).
[55]Y. M. Sheikh and C. Djerassi, *Tetrahedron* **30**, 4095 (1974).
[56]E. Fattorusso, S. Magno, C. Santacroce and D. Sica, *Gazz. Chim. Ital.* **104**, 409 (1974).