Marine polyether triterpenes

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1 Introduction

The traditional sources of bioactive compounds have been terrestrial plants and microorganisms which are easily obtainable and readily explored. In comparison, the sea has been scarcely studied. However since the seventies, as a consequence of the advance of technology, the sea has become an area of priority attention due to the structural diversity and pharmacological potential presented by the molecules isolated from this environment.[1]

One of the most interesting groups of marine natural products is formed by polyether compounds, which from the structural point of view present a great diversity of ring sizes, and from the biological point of view exhibit strong activities. Their biogenetic origins are found in derivatives of fatty acids or in terpenoid compounds from squalene. The present review is dedicated to this second group, and attempts to offer an overview of their isolation and structural determination and explain what synthetic chemistry has achieved and summarise the investigation into their biological activity focused on the exhibited cytotoxicity. These metabolites have been identified in red algae of the genus *Laurencia* and in sponges mainly of the Axinellidae family. All known triterpenes, isolated from algae or sponges, are squalene-derived polyethers which, in almost all cases, consist of two separate ring systems which have led to a series of characteristic compounds. The present article is therefore structured in two parts, the first dedicated to polyethers isolated from algae and the second to polyethers isolated from sponges, in order to relate the chemical structures in each group.

2 Polyether triterpenoids from algae

2.1 Isolation and structure determination

The red algae of the genus *Laurencia* (Rhodomelaceae) are known to produce interesting secondary metabolites. This interest is justified by the high number and variety of backbones found. Such diversity is a consequence of the halogenated nature of these substances, one of their principal characteristics being that it easily permits intramolecular processes that alter their structures.

The characteristic secondary metabolites of *Laurencia* can be divided into two groups according to their biogenetic origin: nonterpenoids and terpenoids.[2–4] The first large group of metabolites isolated from these algae contains nonterpenoid C-15 acetogenins derived from the metabolism of fatty acids. Sesquiterpenoids are the most abundant in the terpenoids group, and they have been isolated in a high number and great diversity. However, the list of examples of diterpenoids and triterpenoids is short.

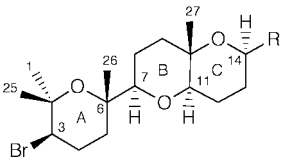
The polyoxygenated triterpenoids from *Laurencia* can be divided on the basis of their structural characteristics, into three different types: (a) compounds that possess a dioxabicyclo-[4.4.0]decane ring system; (b) compounds with a dioxabicyclo[5.4.0]undecane ring system and (c) compounds with symmetric elements.

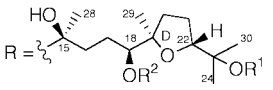
2.1.1 Compounds with a dioxabicyclo[4.4.0]decane ring system. The first example of a marine squalene-derived polyether triterpenoid possessing a dioxabicyclo[4.4.0]decane, B–C ring was reported from *Laurencia thyrsifera* collected off New Zealand by Blunt *et al.* in 1978. This highly oxygenated derivative, thyrsiferol, was assigned the structure **1** on the basis of X-ray crystallographic analysis of thyrsiferyl 18-acetate (**2**) but the absolute stereochemistry was not defined.[5,6] Limited biological testing of the crude samples of these compounds was accomplished and indicated that these compounds were inactive against *Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Aspergillus niger* and *Saccharomyces cerevisiae*.

The second example of this series, dehydrothyrsiferol (**3**), was identified six years later from specimens of *L. pinnatifida* collected in the Canary Islands, Spain.[7] Its structure, which presented a double bond between C-15–C-28, was secured by chemical transformation into thyrsiferol (**1**).

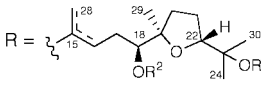
A new cyclic ether, thyrsiferyl 23-acetate (**4**), was isolated from *L. obtusa* collected in Teuri Island, Japan. Its structure was determined by the corresponding hydrolysis which yielded thyrsiferol (**1**).[8] Since the crude extract exhibited a strong cytotoxic property (ED_{50} = 0.18 μg mL^{-1}) against P-388 lymphoid neoplasm cells, the pure compound and its acetate derivatives thyrsiferyl 18,23-diacetate (**5**) and thyrsiferyl 18-acetate (**2**) were tested. Compound (**4**), thyrsiferyl 23-acetate, proved to be a powerful cell growth inhibitor (ED_{50} = 0.3 ng mL^{-1}) (Table 1).[9]

Venustatriol (**6**) was the subsequent compound to be described and was isolated from a crude extract of *L. venusta* that displayed significant activity against the vesicular stomatitis virus (VSV) and *Herpes simplex* type 1 (HSV-1).[10] Its structure and absolute configuration of the ten chiral centres were determined by X-ray analysis and the latter also permitted the correct assignment of the configuration of thyrsiferol (**1**).

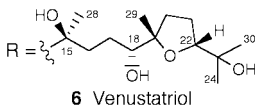




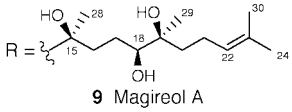
1 Thyrsiferol $R^1 = R^2 = H$
2 Thyrsiferyl 18-acetate $R^1 = H$, $R^2 = Ac$
4 Thyrsiferyl 23-acetate $R^1 = Ac$, $R^2 = H$
5 Thyrsiferyl 23,18-diacetate $R^1 = R^2 = Ac$



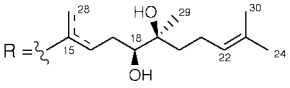
3 $\Delta^{15,28}$Dehydrothyrsiferol $R^1 = R^2 = H$
7 $\Lambda^{15,28}$15(28)-Anhydrothyrsiferyl diacetate $R^1 = R^2 = Ac$
8 $\Delta^{15,16}$15-Anhydrothyrsiferyl diacetate $R^1 = R^2 = Ac$



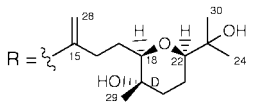
6 Venustatriol



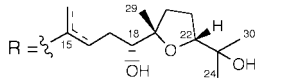
9 Magireol A



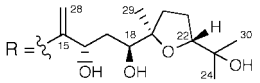
10 $\Lambda^{15,28}$Magireol B
11 $\Delta^{15,16}$Magireol C



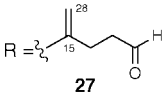
13 Isodehydrothyrsiferol



17 $\Lambda^{15,28}$Dehydrovenustatriol
18 $\Delta^{15,16}$15,16-Dehydrovenustatriol



20 16-Hydroxydehydrothyrsiferol



27

28

Both molecules share the same configuration for the asymmetric centres as 3*R*, 6*S*, 7*R*, 10*S*, 11*R*, 14*R*, and 15*S* and rings A and B possess spatial disposition as chairs, whereas the C ring is forced to adopt a boat conformation to avoid an otherwise unfavorable 1,3-diaxial interaction between substituents in the alternative chair conformation (Fig. 1). The essential difference resides in the tetrahydrofuran substituent pattern. The configuration of the C-18 and C-19 atoms in thyrsiferol (**1**) are the opposite of those in venustatriol (**6**), while at the C-22 position they remain the same (Scheme 1).

These marine algae squalene polyethers generated a high degree of interest due to their original structures, possessing potent cytotoxic effects, and various synthetic efforts were directed towards producing these compounds.

The first synthetic attempts consisted of the preparation of the central fragment (rings B and C) using a mercuricyclisation–oxidative demercuriation strategy.[11] Thereinafter the construction of a tricyclic bromoether such as thyrsiferol (**1**) and venustatriol (**6**) A, B–C rings were reported with good stereochemical control.[12,13] Finally, both compounds and thysiferyl 23-acetate (**4**) were totally synthesised from trivial compounds by an enantioselective and convergent routes.[14–19]

Five new cytotoxic compounds were isolated from a neutral extract of *L. obtusa* 15(28)-anhydro thyrsiferyl diacetate (**7**), 15-anhydrothyrsiferyl diacetate (**8**), magireol A (**9**), magireol B (**10**) and magireol C (**11**).[20] Their structures were determined by spectroscopic analysis and chemical correlation. Compound **5** was treated with thionyl chloride and produced a mixture of **7** and **8**, and the confirmation of the structure of magireol A (**9**) was carried out by its conversion into thyrsiferol (**1**) using epoxidation with *m*-chloroperbenzoic acid and treatment with toluene-*p*-sulfonic acid. These compounds showed strong activity in the assay with the P-388 cells line (Table 1).[9,20]

Another pentacyclic compound, callicladol (**12**),[21] was isolated from *L. calliclada* collected on the Vietnamese coasts. Its structure possesses the A-B-C ring moiety similar to thyrsiferol and its congeners, with a β-secondary hydroxy group at C-5. The relative stereochemistries were determined by NOE correlations and *J* values indicated that all methine protons on the A-B-C-rings were oriented in the axial direction, and the absolute configuration at C-5 was assigned as *S*, using advanced Mosher's methods.[22] In this compound no NOE was detected between the other ether linkages, thus suggesting that the stereochemistries of the two oxolane D-E rings were also *trans*. Callicladol (**12**) displayed a cytotoxic activity *in vitro* against P-388 murine leukemia cells with IC_{50} of 1.75 μg mL^{-1}.

It is unquestionable that *L. viridis*, a new species described from specimens collected around the Canary Islands, in Macaronesia, is the most prolific producer of this type of metabolite. It is an annual plant that grows in the lower intertidal zone, intermingled with other turf algae. It grows rapidly during the winter–spring months and decays in late summer.[23] From this alga nine new examples of thyrsiferol or venustatriol congeners have been isolated. Their structures were determined through the interpretation of spectral data and the relative stereochemistry is proposed on the basis of ROESY and NOEDIFF data. Together with dehydrothyrsiferol (**3**), two isomers, isodehydrothyrsiferol (**13**) and 10-*epi*-dehydrothyrsiferol (**14**) were isolated from the acetone extract.[24] In compound (**13**) the D-ring has changed to an oxane ring system instead of the oxolane ring system present in compound **3**. In the other isomer **14** the changes are located in the fused B-C ring which must be *cis*-fused with a flexible conformation, instead of the *trans*-fused system present in all metabolites previously isolated.

In a later study, thyrsenol A (**15**) and B (**16**) from the chloroform–methanol extract were isolated.[25] These compounds possess an unusual enol–ether system in ring C at carbons C-13–C-14 together with a diol system at carbons C-15–C-28. The relative stereochemistry at C-15 was established as *R** in thyrsenol A (**15**) and as *S** in thyrsenol B (**16**). The rest of the chiral centres of these molecules were identical to those observed in dehydrothyrsiferol (**3**).

Five new metabolites closely related to thyrsiferol (**1**) and venustatriol (**6**) were also described.[26] Dehydrovenustatriol (**17**) and 15,16-dehydrovenustatriol (**18**) showed a double bond at C-15–C-28 and a trisubstituted double bond between C-15–C-16 with a (*Z*)-configuration respectively. In 10-*epi*-15,16-dehydrothyrsiferol (**19**) the presence was clearly established of an (*E*)-trisubstituted double bond between carbon C-15–C-16, instead of between carbons C-15–C-28 in **14**; and in 16-hydroxydehydrothyrsiferol (**20**) the presence of a secondary hydroxy group at C-16 with relative configuration *S** could be established in concordance with the spectroscopical data.

The fifth and last compound isolated in this study and the most interesting from the biogenetic point of view was predehydrovenustatriol acetate (**21**). It structure showed that the B-C ring as well as the side chain were identical with those of dehydrovenustatriol 18-acetate. The important feature to emphasise was the absence of the A-ring and the bromine atom in the position C-3 together with the presence of a trisubstituted double bond between the carbon C-2–C-3.

Biological assays of these pure isolates were undertaken, making use of *in vitro* bioassays focusing on cytotoxic activities and the results are summarised in Table 1.

2.1.2 Compounds with a dioxabicyclo[5.4.0]undecane ring system. Enshuol (**22**), isolated from *L. omaezakiana*, was the first example of a new class of pentacyclic bromotriterpenoids.[27] Its structure is formed by a 2,8-dioxabicy-

Table 1 IC_{50} from biological assays of the pure polyether squalene derivatives isolated from algae and sponges

Compounds		IC_{50}							
		Cell lines							
		P-388		A-549		HT-29		MEL-28	
		μg mL^{-1}	μM	μg mL^{-1}	μM	μg mL^{-1}	μM	μg mL^{-1}	μM
Metabolites from *Laurencia* algae:									
(**1**)	Thyrsiferol	0.01	0.016	10.0	16.53	10.0	16.53		
(**2**)	Thyrsiferyl 18-acetate	0.30	0.47						
(**3**)	Dehydrothyrsiferol	0.01	0.017	2.5	4.26	2.5	4.26	5	8.52
(**4**)	Thyrsiferyl 23-acetate	0.0003	0.00047						
(**5**)	Thyrsiferyl 18,23-diacetate	0.52	0.75						
(**7**)	15(28)-Anhydrothyrsiferyl diacetate	0.05	0.074						
(**8**)	15-Anhydrothyrsiferyl diacetate	0.10	0.148						
(**9**)	Magireol A	0.03	0.052						
(**10**)	Magireol B	0.03	0.052						
(**11**)	Magireol C	0.03	0.052						
(**12**)	Callicladol	1.75	2.89						
(**13**)	Isodehydrothyrsiferol	0.01	0.017	2.5	4.26	2.5	4.26	2.5	4.26
(**14**)	10-epi-Dehydrothyrsiferol	1.00	1.70	5.0	8.52	5.0	8.52	5.0	8.52
(**15**)	Thyrsenol A	0.25	0.40	> 1.0	> 1.62	> 1.0	> 1.62	> 1.0	> 1.62
(**16**)	Thyrsenol B	0.01	0.016	> 1.0	> 1.62	> 1.0	> 1.62	> 1.0	> 1.62
(**17**)	Dehydrovenustatriol	0.01	0.017	2.5	4.26	2.5	4.26	2.5	4.26
(**18**)	15,16-Dehydrovenustatriol	0.25	0.43	2.5	4.26	2.5	4.26	2.5	4.26
(**19**)	10-epi-15,16-Dehydrothyrsiferol	0.50	0.85	2.5	4.26	1.2	2.04	2.5	4.26
(**20**)	16-Hydroxydehydrothyrsiferol	0.50	0.83	1.2	1.99	1.2	1.99	1.2	1.99
(**21**)	Predehydrovenustatriol acetate	1.20	2.18	2.5	4.54	5.0	9.09	2.5	4.54
(**27**)		> 1.00	> 2.26	> 1.00	> 2.26	> 1.00	> 2.26		
(**28**)		> 2.00	> 4.96	> 2.00	> 4.96	> 2.00	> 4.96		
Metabolites from sponges of the Axinellidae family:									
(**50**)	Sodwanone G	1	2.0	0.1	0.2	1	2.0	1	2.0
(**51**)	Sodwanone I	9.8	20.0	9.8	20.0	9.8	20.0	9.8	20.0
(**47**)	Sodwanone M	1	2.0		—		—		—

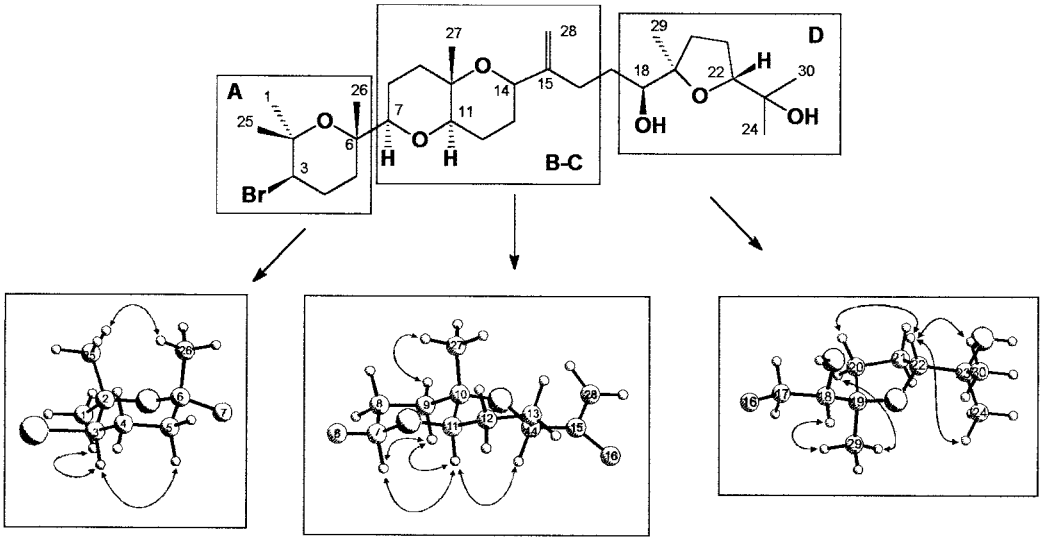
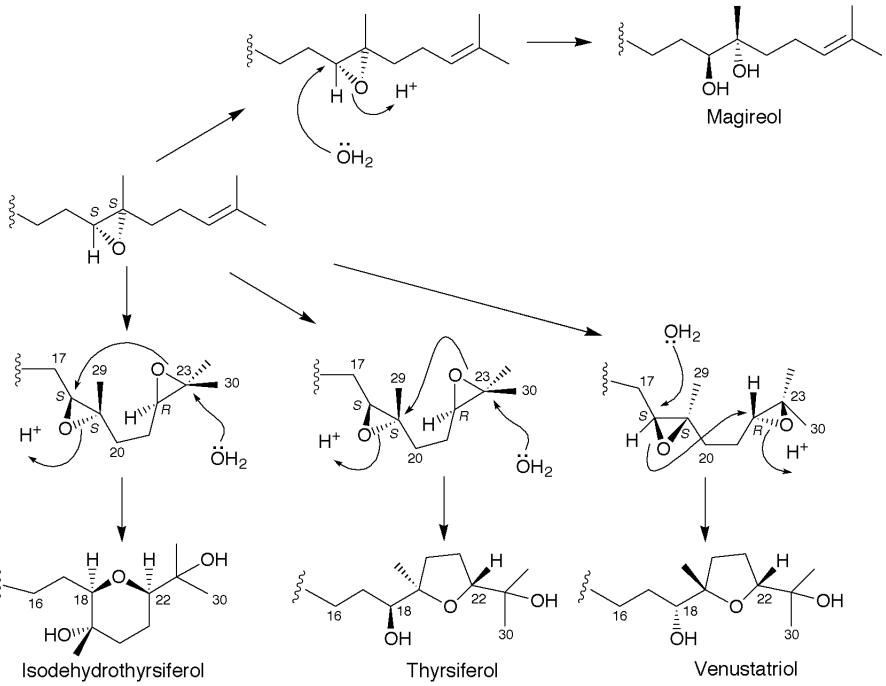


Fig. 1 Spatial disposition and more significant NOE correlations in dehydrothyrsiferol (**3**), similar in rings A, B and C to venustatriol (**6**).

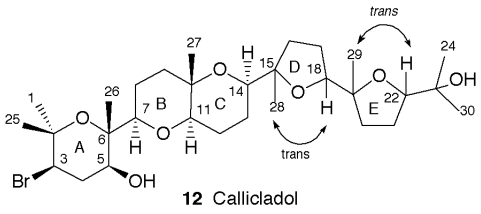
clo[5.4.0]undecane ring (A-B ring) and three isolated oxolane rings (C-D-E rings). The stereochemistry about the 2,8-dioxabicyclo[5.4.0]undecane ring as 3*R*, 6*R* and 7*S* was determined by the NOESY and NOE difference spectra and application, as in the previous case, of advanced Mosher's methods with the compound obtained from treatment of **22** with zinc powder and acetic acid.[22] No NOE was detected between the other ether linkages, thus suggesting that the stereochemistries of the three oxolane rings were *trans*.

In this group must be included aurilol (**23**)[28] which was isolated from a sea hare *Dolabella auricularia* (order Aplysiacea, family Aplysiidae) and it is possible that the origin is to be found in the diet of the mollusc. The structure of **23** presents an A–B ring with a 2,8-dioxabicyclo[5.4.0]undecane system and two isolated oxolane rings C and D. The stereochemistry was proposed on the basis of NOESY correlations, comparing coupling constants with simple stereoisomer models and the preparation of (*R*) and (*S*)-bisMTPA. Thus the absolute stereochemistry of the five stereocentres was determined to be 3*R*, 6*R*, 7*S*, 10*R* and 22*R*. Though no NOE was detected between the other ether linkages, it is suggested that the stereochemistries of the two oxolane rings were *trans* in the C ring and *cis* in the D ring. Aurilol (**23**) exhibited cytotoxicity against HeLa S_3 cells (human cervix carcinoma) with an IC_{50} of 4.3 μg mL^{-1}.

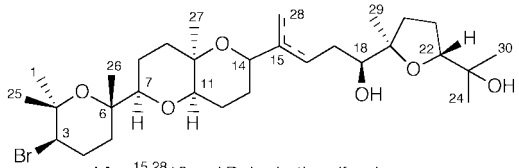
2.1.3 Symmetric compounds. The *meso*-compound teurilene (**24**) was isolated from *L. obtusa* collected in Teuri Island



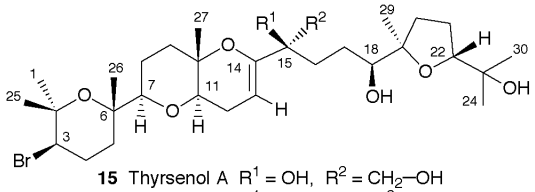
Scheme 1



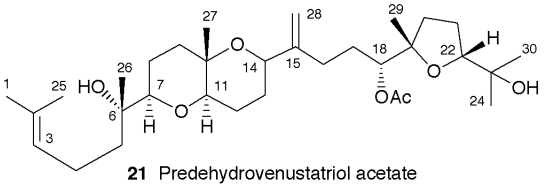
12 Callicladol



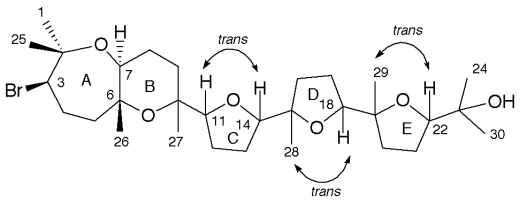
14 $\Delta^{15,28}$10-*epi*-Dehydrothyrsiferol
19 $\Delta^{15,16}$10-*epi*-15,16-Dehydrothyrsiferol



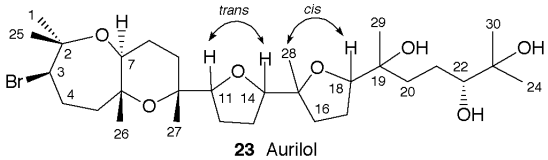
15 Thyrsenol A R^1 = OH, R^2 = CH_2–OH
16 Thyrsenol B R^1 = CH_2–OH, R^2 = OH



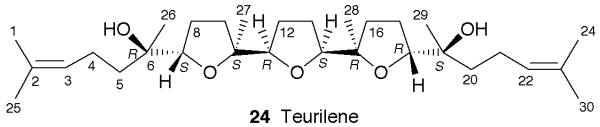
21 Predehydrovenustatriol acetate



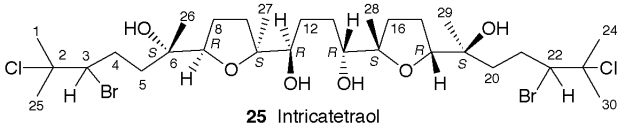
22 Enshuol



23 Aurilol



24 Teurilene



25 Intricatetraol

together with thyrsiferyl 23-acetate (**4**).[8] The structure of this metabolite was determined by X-ray methods and possesses three linked tetrahydrofuran units in the centre of the molecule with eight chiral centres and C_s symmetry. Teurilene (**24**), an inactive compound against the P-388 cells line, attracted special interest in its synthesis and conformational properties. The strategy utilised by Shirahama *et al.* in this work is interesting in that optically pure fragments are used to provide an optically inactive product.[19,29,30]

A unique halogenated example with chlorine atoms and C_2 symmetry feature, intricatetraol (**25**), was isolated from the red alga *L. intricata*. Its structure was deduced from spectral, chemical and biogenetic evidence.[31] Treatment of **25** with zinc and acetic acid resulted in dehalogenation to afford a symmetrical vinyl compound closely resembling the *meso* compound teurilene (**24**). The positions of four halogen atoms were determined by spectral and chemical methods.[32] The relative stereochemistry about the oxolane rings was defined by NOE difference spectra, and the absolute configuration of the hydroxy groups at C-11 and C-14 was established by application of the advanced Mosher's methods.[22]

2.2 Biogenetic considerations

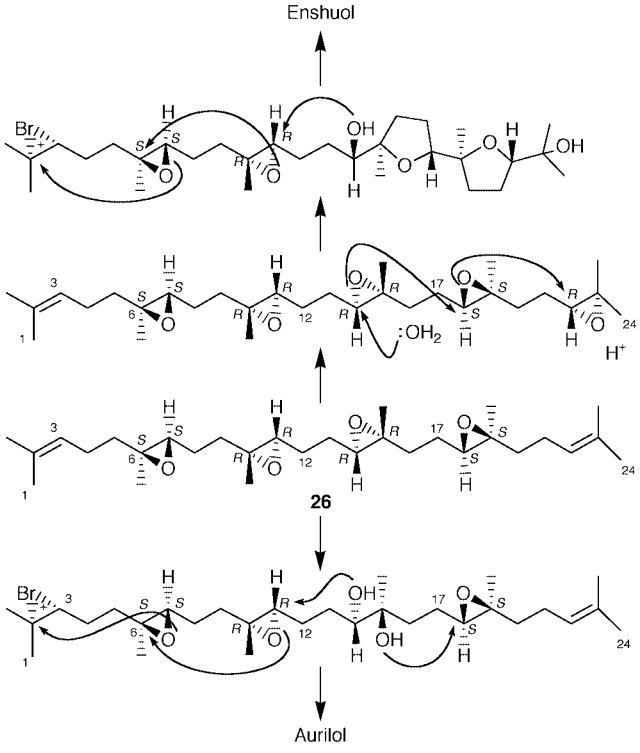
From a biogenetic viewpoint, the polyoxygenated squalene-derived ethers isolated from *Laurencia* species may arise from a common precursor the (10*R*,11*R*)-squalene 10,11-epoxide isolated from *Laurencia okamurai*.[33] This compound can evolve to (6*S*,7*S*,10*R*,11*R*,14*R*,15*R*,18*S*,19*S*) squalene tetraepoxide (**26**) as a common intermediate, which has not yet been found. From this intermediate three routes can be proposed: the first is to thyrsiferol (**1**), venustatriol (**6**) and its congeners that possess a dioxabicyclo[4.4.0]decane B–C ring system, the second is to compounds that possess a 2,8-dioxabicyclo[5.4.0]undecane A–B ring system and the third implies the genesis of symmetric compounds such as teurilene (**24**).

In thyrsiferol (**1**) or venustatriol (**6**) and its congeners, from the biogenetic viewpoint, it is possible to observe different processes of cyclisation. Ring D is possibly biosynthesised from the diepoxide fragment 18*S*,19*S*,22*R*, by protonation to (18*S*,19*S*)-epoxide followed by hydroxylation at C-23 to yield the oxolane ring in the thyrsiferol series or the oxane ring in isodehydrothyrsiferol (**13**). When the process is started by protonation in (22*R*)-23-epoxide followed by hydroxylation at C-18, the venustatriol series is produced as illustrated in Scheme 1.

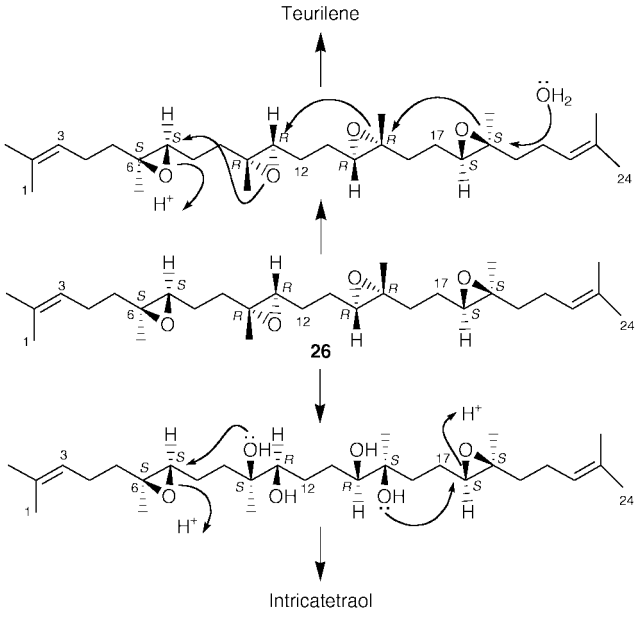
On the other hand, initially the formation of the A–B–C system was believed to be through a concerted cyclisation of three epoxides after formation of a bromonium ion at C-2–C-3. However, as a consequence of the identification of predehydrovenustatriol acetate (**21**) which lacks the bromine and ring A, and possesses a double bond between the carbons C-2–C-3, and considering the changes at the stereochemistry in C-10 in the compounds 10-*epi*-dehydrothyrsiferol (**14**) and 10-*epi*-15,16-dehydrothyrsiferol (**19**) and the presence in thyrsenol A (**15**) and B (**16**) of the enol–ether in carbon C-13–C-14 or the β-hydroxy group at C-5 in callicladol (**12**), the classic biogenetic proposal for these metabolites would be in contradiction and it would be more convenient to assume that cyclisation occurs through a sequential process.

The second group is formed by the compounds with a dioxabicyclo[5.4.0]undecane ring system. Its biogenetic proposal is also based on the tetraepoxide intermediate **26**. In these compounds the process starts with an enzymatic protonation to (14*R*,15*R*)-epoxide and evolves following the pathway in Scheme 2 toward the products enshuol (**22**), proposed with all *trans*-relationships between the three oxolane ether linkages,[27] and aurilol (**23**) which was described with a *trans*-relationship between H-11 and H-14, and a *cis*-relationship between H-18 and H_3-28.[28]

For the last group considered, teurilene (**24**) and intricatetraol (**25**), Suzuki *et al.*[31] have proposed a biogenesis. Teurilene (**24**) may be derived from squalene tetraepoxide (**26**) through initial enzymatic protonation to (6*S*,7*S*)-epoxide followed by cyclisation and hydroxylation at C-19. In **25** the same origin is assumed, that is, from **26**, by protonation to (6*S*,7*S*)-epoxide and subsequent cyclisation and hydroxylation at C-11 to give a half (6*S*,7*R*,10*R*,11*S*)-configuration with a *trans*-relationship between H-7 and H_3-27. The other half may be generated by a 14,15-diol intermediate which seems to be initially formed by protonation and hydroxylation to the 14,15-epoxide. The reaction of the hydroxy group at C-15 with the epoxide at C-18 would give this half with a *cis*-relationship between H-18 and H_3-28. Subsequent halogenation produces the compound **25** (Scheme 3).



Scheme 2



Scheme 3

2.3 Biological activity

From the moment in which thyrsiferyl 23-acetate (**4**) was isolated and found to exhibit strong cytotoxicity, the biological assay of this type of compound was focused on evaluating this property. Cytotoxic effects were evaluated with cultured cell lines of P-388 (murine lymphoid neoplasm), A-549 (human lung carcinoma), HT-29 (human colon carcinoma) and MEL-28 (human melanoma). These screening procedures established the cytotoxic activity of these compounds, showing them to possess a potent and selective activity against P-388 cells. The results of the biological assay are summarised in Table 1.

A study of structure–activity relationships was accomplished with the metabolites isolated from *L. viridis*.[34] This study started with a process of molecular simplification yielding by oxidative fragmentation the compounds **27** and **28**. These modifications reduced considerably their cytotoxicity and selectivity against the leukaemia suspension cells. A possible explanation of these facts suggests the necessary presence of the flexible chain around carbons C-15 to C-24 and the different

conformations of the analogues on the basis of the correlations obtained between the arrangements of the flexible chain and IC_{50} values. The most active metabolites possess similar arrangements to dehydrothyrsiferol (**3**) (IC_{50} = 0.01 μg mL^{-1}), and when the direction of the chain is turned in both directions the power of the cytotoxic activity is reduced progressively, as occurred with thyrsenol A (**15**) (IC_{50} = 0.25 μg mL^{-1}) and 10-*epi*-dehydrothyrsiferol (**14**) (IC_{50} = 1.0 μg mL^{-1}) (Fig. 2).

Thyrsiferyl 23-acetate (**4**) has been shown to potently and specifically inhibit serine/threonine protein phosphatase type 2A (PP2A) with IC_{50} values of 4–16 μM, depending on the enzyme concentration.[35] This compound did not affect activity of protein phosphatase type 1 (PP1), 2B (PP2B), 2C (PP2C), or protein tyrosine phosphatases (PTP) up to 1 mM. Furthermore, it inhibited PP2A activity in a crude extract of a human T cell line, Jurkat cell, as well as the purified catalytic subunit. This work suggests that thyrsiferyl 23-acetate (**4**) can be, together with okadaic acid, microcystin LR, calyculin A and tautomycin, a useful tool to study the cellular process mediated by phosphatases.

Venustatriol (**6**) has been described with activity against the vesicular stomatitis virus (VSV) and *Herpes simplex* type 1 (HSV-1).[10] But the biological assays were accomplished with a crude extract of *L. venusta* in which were also identified thyrsiferol (**1**) and thyrsiferyl 23-acetate (**4**), and advanced trials with the pure product have not yet been published.

Mechanisms of growth inhibition by dehydrothyrsiferol (**3**) were investigated in a sensitive and an MDR^+ human epidermoid cancer cell lines.[36] This compound was found to circumvent multidrug resistance mediated by P-glycoprotein and might therefore be an interesting candidate for development in future clinical trials. By treatment of KB cells with compound **3**, the cell cycle analysis revealed an accumulation in S-phase and it did not cause any obvious increase in either apoptosis or necrosis. Taken together, no parallels in the phenomena of growth inhibition could be detected.

The cytotoxicity of dehydrothyrsiferol (**3**) against three human breast cancer cell lines, T47D (IC_{50} of 13.5 μM), ZR-75 (IC_{50} of 16.0 μM) and Hs578T (IC_{50} of 18.9 μM), was examined and compared with the chemotherapeutic compound doxorubicin and the mitosis-inhibitor colchicine and proved that this metabolite does not modulate P-glycoprotein (P-gp) mediated drug transport. Therefore, it could be used in P-gp expressing cancer cells without interference.[37]

Some of the known terpenoids (*e.g.* paclitaxel, docetaxel, *etc.*) are already in clinical use or clinical trials, but none of them were derived from the marine environment. These marine polyether terpenoids might thus represent a source of interesting drugs mediating unconventional antineoplastic effects.

3 Polyether triterpenoids from sponges

3.1 Isolation and structure determination

The terpenoids are to date the most abundant nonsteroidal secondary metabolites isolated from marine sponges. Many interesting sesqui-, di-, and sesterpenes have been characterised from these animals,[1] but until the isolation of the sipholanes from the Red Sea sponge *Siphonochalina siphonella*, order Haploscheridae, family Halichonidae, aside from squalene in 1981,[38] no other triterpenes had been reported. More recent ones have been mainly isolated from three other sponges *Axinella weltneri*, *Raspaciona aculeata*, and *Ptilocaulis spiculifer*, all of the Axinellidae family, collected from the Indo Pacific, Mediterranean and Red Seas. These metabolites consist, in almost all cases, of two separate cyclic systems and we have carried out their classification in accordance with the type of linker that connects those systems: (a) an ethylene bridge that generally connects two *trans*-decahydrobenzooxepines or a *trans*-decahydrobenzooxepine with another cyclic system; (b) a modified or rearranged bridge and (c) a butylene bridge.

3.1.1 Compounds with an ethylene bridge. The sipholanes possess two separate uncommon bicyclic systems, octahydroazulene and *trans*-decahydrobenzooxepine, linked to each other by an ethylene bridge. As far as we know, just one of these metabolites, sipholenone B (**29**), isolated from the sponge *Siphonochalina siphonella*,[39] has presented a polyetheric structure. This was determined by chemical correlations with related compounds sipholenol (**30**) and sipholenone A (**31**),[39] but those were lately corrected in their absolute configuration by Kakisawa *et al*. by applying high field NMR to Mosher's methods.[22,40] Therefore we propose that the structure of sipholenone B should be **29** instead of the reported enantiomer.

In 1990, structurally related to those sipholane metabolites, from the liposoluble extracts of *Raspaciona aculeata* was isolated the first and major representative of a new triterpenoid skeleton class, raspacionin (**32**),[41] which exhibited frameworks characterised by two *trans*-perhydrobenzooxepine systems linked by an equatorial ethylene bridge. *Raspaciona aculeata* is a red encrusting Mediterranean sponge and offers some intriguing goads to chemical investigations. Firstly, its taxonomic classification was a matter of discussion. In fact, *R. aculeata* belongs to the family Paspailiidae which alternatively has been placed into the order Poecilosclerida or Axinellida, on the basis of skeletal features. Secondly, this sponge seems to display some allelochemical activities since it is completely devoid of epibiotic organisms. The structure of raspacionin (**32**) was suggested by analysis of its spectral data and the relative

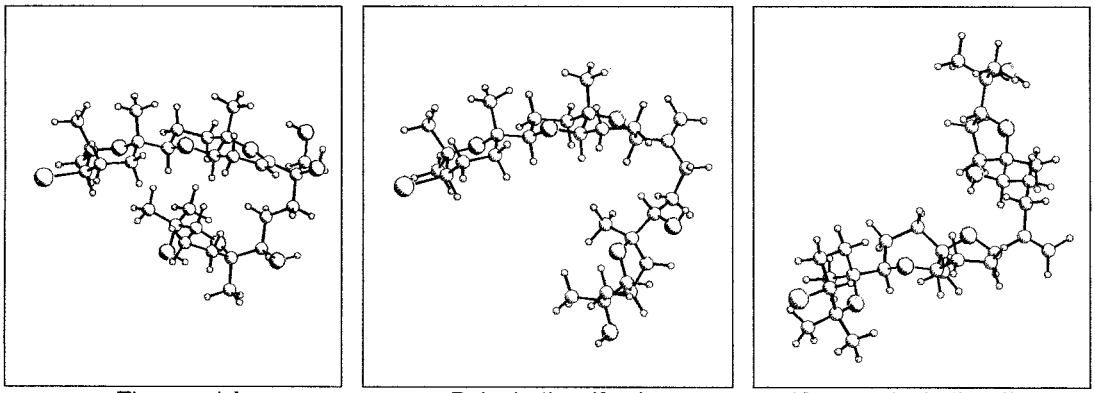
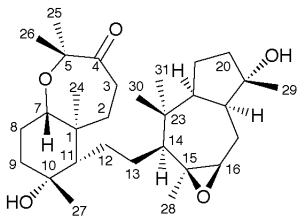
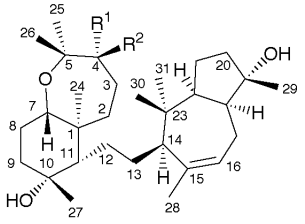


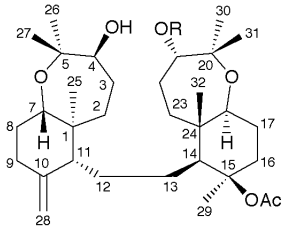
Fig. 2 Stereo view of proposed 3D structures and IC_{50} values for representative compounds isolated from *L. viridis*.



29 Sipholenone B



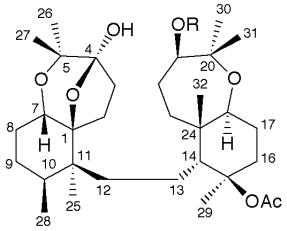
30 Sipholenol $R^1 = H$, $R^2 = OH$
31 Sipholenone A $R^1 = R^2 = O$



32 Raspacionin R = Ac
35 21-Deacetyl-raspacionin R = H

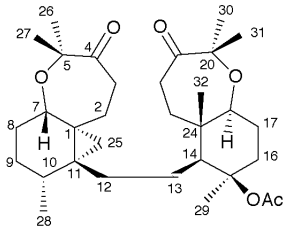
stereochemistry was secured by single-crystal X-ray diffraction analysis,[41,42] but the absolute stereochemistry was not determined until 1993 by application of high field NMR to Mosher's methods with α-methoxy-α-trifluoromethylphenylacetic (MTPA) esters.[43]

Co-occurring in the same sponge and all based on the same triterpenoid skeleton of compound **32** have been isolated other polyetheric metabolites, raspacionin A (**33**),[44] raspacionin B

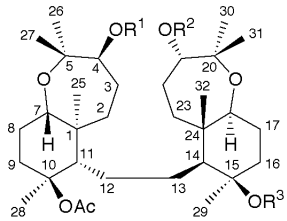


33 Raspacionin A R = Ac
43 21-Deacetyl-raspacionin A R = H

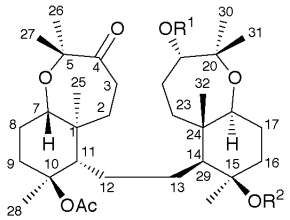
(**34**),[45] and a further eight **35**–**42** minor components.[46] The main differences between the structure of raspacionin (**32**) and compound **33** are the absence of the *exo*-methylene system at C-10–C-28 and the presence in the perhydrobenzooxepine system of an oxygen between C-4 and C-1 which, bearing in mind the biogenetic origin from a squalene precursor, should induce a 1,2-shift of the methyl from C-1 to C-11 leading to a rearranged triterpenoid skeleton **33**. This was unambiguously determined by X-ray diffraction studies of its deacetyl derivative **43**. The absolute stereochemistry of both compounds was proposed, by applying Mosher's methods, as *R* at C-21 for **43** and **33**. The structure and relative stereochemistry of raspacionin-B (**34**) were elucidated by means of spectral methods, mainly 1D and 2D NMR, suggesting a structure related to raspacionin (**32**) but displaying two ketone groups at C-4 and C-21. This metabolite is also characterised by the presence of a cyclopropane ring as was established by HOHAHA and HMBC experiments. Its absolute stereochemistry was proposed by comparison of its CD curve with those of related compounds **33** and **31**. With regard to the minor raspacionins, their structures display different functionalisations at carbons 4, 10, 15 and 21.[46] Their full NMR characterisation has led to the rationalisation of the effects of certain substituents on the chemical shifts of atoms in the perhydrobenzooxepine system. The absolute stereochemistry of these compounds was assumed to be presumably the same as that of the other raspacionins on the basis of the display of positive CD maximum at *ca.* 302 nm for compounds **37**, **38**, **39**,



34 Raspacionin B

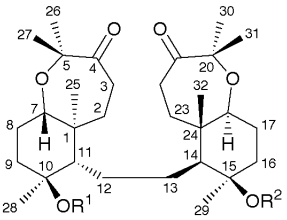


36 10-Acetoxy-21-deacetyl-28-hydroraspacionin
$R^1 = R^2 = H$, $R^3 = Ac$
40 10-Acetoxy-4-acetyl-15-deacetyl-28-hydroraspacionin
$R^1 = R^2 = Ac$, $R^3 = H$



37 10-Acetoxy-21-deacetyl-4-oxo-28-hydroraspacionin $R^1 = H$, $R^2 = Ac$
38 10-Acetoxy-15,21-dideacetyl-4-oxo-28-hydroraspacionin $R^1 = R^2 = H$
39 10-Acetoxy-15-deacetyl-4-oxo-28-hydroraspacionin $R^1 = Ac$, $R^2 = H$

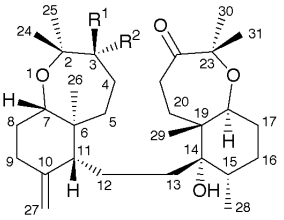
41 and **42** due to a ketone moiety, that suggested an absolute stereochemistry identical with those of the 4-oxo-derivative of raspacionin (**32**), the 21-oxo-derivative of raspacionin A (**33**), and raspacionin B (**34**).[45]



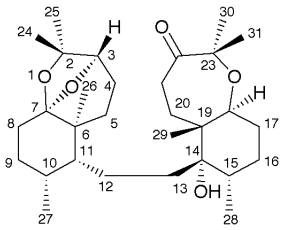
41 10-Acetoxy-15-deacetyl-4,21-dioxo-28-hydroraspacionin
$R^1 = Ac$, $R^2 = H$
42 10-Acetoxy-4,21-dioxo-28-hydroraspacionin $R^1 = H$, $R^2 = Ac$

Closely related to the above metabolites, a novel group of triterpenoids named sodwanones has been isolated from the sponge *Axinella weltneri*. The sodwanones possess at least one perhydrobenzooxepine system derived from one half of the squalene precursor and a variety of other systems obtained from the second half of the squalene, that so far belong to three different carbon skeletons. One of them, constituted by the sodwanones D (**44**), E (**45**), F (**46**), K (**47**), and M (**48**), comprises also two perhydrobenzooxepine systems as raspacionins and those metabolites can be classified within the same class.

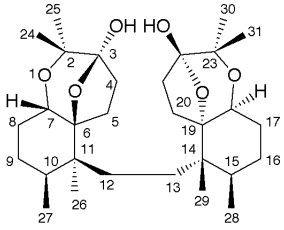
Sodwanones D (**44**), E (**45**) and F (**46**),[47] were isolated from the Indo-Pacific specimen of these sponges. The structures were initially determined on the basis of their NMR data but like the structure elucidations of compounds **45** and **46** needed further clarification. Thus, X-ray diffraction of those metabolites was also carried out.[48] The structure of sodwanone D (**44**) was proposed to comprise two characteristic perhydrobenzooxepine



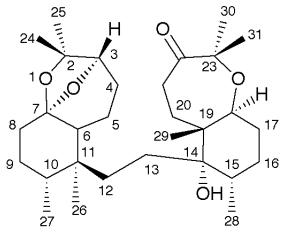
44 Sodwanone D $R^1 = R^2 = O$
47 Sodwanone K $R^1 = H$, $R^2 = OH$



45 Sodwanone E



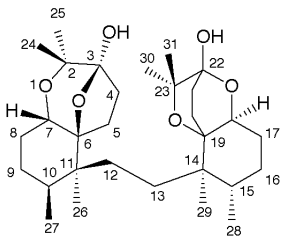
46 Sodwanone F



48 Sodwanone M

systems 3-oxo-2,2,6- and 22-oxo-19,23,23-trimethylated oxepanes. Sodwanone E (**45**) by NMR data comparison with compound **44** suggested that the main difference between these compounds was the presence of an internal ketal group, C-3–C-7. The relative stereochemistry of this metabolite was suggested by NOEs and the X-ray analysis allowed confirmation of the previous suppositions as well as establishing the relative stereochemistry of the two halves pointing out the slight twisting of the left half oxepane ring. Comparison of the NMR data of sodwanone F (**46**) with those of raspacionin A (**33**) clearly led to the identification of half of the structure as the same as that of the corresponding left half in compound **33**. Moreover, the second half turned out to be similar to the first one, differing in the stereochemistry at C-15, as was ascertained by X-ray diffraction analysis.[48]

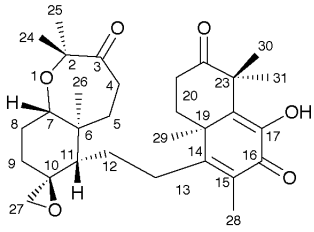
From specimens of *Axinella weltneri* collected in the Comoros Islands was isolated sodwanone D (**44**) together with three new triterpenes, sodwanones M (**48**), K (**47**) and L (**49**).[49] Comparison of spectral data for sodwanone M (**48**), the major



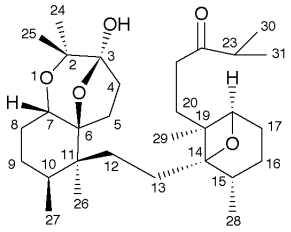
49 Sodwanone L

isolated compound, with those of the earlier reported sodwanones[47,48] suggested that compound **48** had the same perhydrobenzooxepine right half as sodwanone E (**45**) and in the second half the migration of the methyl group, Me-26, from C-6 to C-11 was observed. A similar 1,2-shift was previously described for sodwanone F (**46**)[47] and raspacionin (**32**).[41] The relative configuration assignment of the chiral centres were determined mainly by NOE correlations. Sodwanone M (**48**) was also found to be cytotoxic to P-388 murine leukaemia cells at a concentration of 1 μg mL^{-1}, but was not active against the A-549, MT-29, and MEL-28 human tumor cells (Table 1). The structure of sodwanone K (**47**) was secured by oxidation of the C-3 axial alcohol in the known derivative sodwanone D (**44**).

Two additional sodwanones, G (**50**) and I (**51**), were also isolated from the sponge *Axinella weltneri*.[48] Structure elucidation of compound **50** began by intensive study of its spectro-



50 Sodwanone G

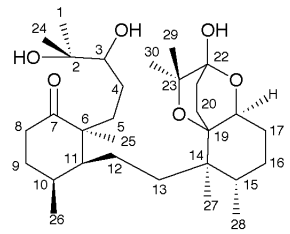


51 Sodwanone I

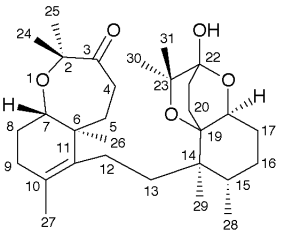
scopic data, and its complete structure as well as its relative stereochemistry were unequivocally established by X-ray diffraction analysis. This indicated that the left half has the same stereochemistry as raspacionin (**32**) and sodwanone D (**44**) whereas the differences were observed for fragment C-8–C-11 containing only seven methyl groups with the eighth one being transformed into the epoxide on the C-10–C-27 methylene. In compound **51**, sodwanone I, the left half of molecule was identical to sodwanone F (**46**), and the other half differed from all other corresponding parts in sodwanones reported to date. The chemical shifts, COSY and HMBC experiments suggested an oxabicyclo[3.1.1]heptane system which was confirmed by a NOESY experiment. Sodwanones G (**50**) and I (**51**) have been found to have cytotoxic activity, being active at 20 μM or less against the four test systems employed: P-388, A-549, MT-29, and MEL-28 cell lines. Sodwanone G (**50**), in contrast to sodwanones M (**48**) and I (**51**), showed high specificity against the human lung carcinoma cell line A-549, ten times less than against the other cell lines tested as shown in Table 1.

Analogously, compound (**49**), sodwanone L, showed the left half to be identical to the left part of sodwanone F (**46**) and the right half possessing an unprecedented dioxabicyclo[2.2.2]octane system that represents a new class of sodwanones. The suggested stereochemistry is relative, and either half could be its enantiomer. Thus, the relationship between the two halves, which are too far away for NOEs, was based on X-ray structures of several sodwanones.

Sodwanones N (**52**), O (**53**) and P (**54**)[50] also consist of two separate ring systems. One specific half for each compound that resulted in the same substituted oxepane ring as sodwanones E (**45**) (no closure to ketal), D (**44**) and M (**48**), respectively, and

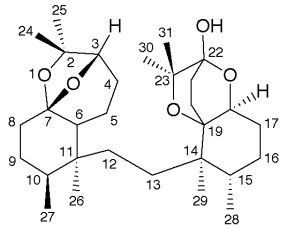


52 Sodwanone N



53 Sodwanone O

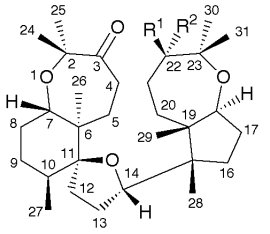
a second common half, C-14 to C-23, has the same tricyclic structure as the "right" half of sodwanone L (**49**). With respect to sodwanone N (**52**) only one of the two central methyl groups of the squalene precursor shifted to its neighboring C-atom, whereas both groups shifted in sodwanone F (**46**). The relative stereochemistry of the two halves in these compounds, which cannot be determined by NOE correlations, due to conforma-



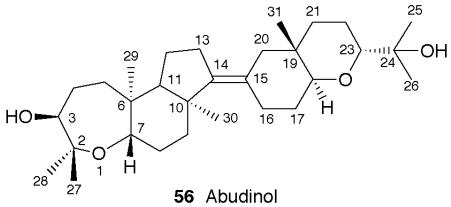
54 Sodwanone P

tional mobility around the C-12–C-13 bond, is believed, with the same reservations, to be the same as that of sodwanone L (**49**) with the corresponding 'left' parts.

3.1.2 Compounds with a modified or rearranged bridge. From *Ptilocaulis spiculifer* collected in the Red Sea have been isolated in addition to several known sodwanones D (**44**), F (**46**) and I (**51**), five new triterpenoids: yardenone (**55**) and abudinol (**56**) from the first collection;[51] and 22-dihydroyardenone (**57**),

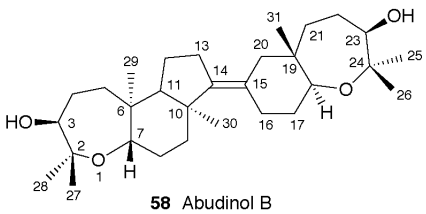


55 Yardenone $R^1 = R^2 = O$
57 22-Dihydroyardenone $R^1 = H$, $R^2 = OH$



56 Abudinol

abudinol B (**58**) and muzitone (**59**) from the second.[50] The structures of compounds **55** and **56** were determined by interpretation of their spectral data and were secured, including their relative stereochemistry, by X-ray diffaction analysis. A comparison of data for **57** with the earlier reported triterpenes suggested a close relationship. Atoms 1 to 19, forming four rings, were identical to those in yardenone (**55**) while the C-atoms of the fifth, a second oxepane ring, differed where a carbonyl has been replaced by an OH-group at C-22, confirmed by Jones oxidation of **57** with aq. $Na_2Cr_2O_7$–acetone to afford yardenone (**55**), the stereochemistry of the 22-OH-group being on the same side of the ring as H-18. Abudinol B (**58**) proved to

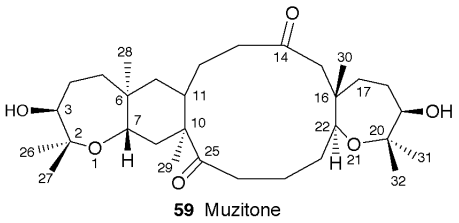


58 Abudinol B

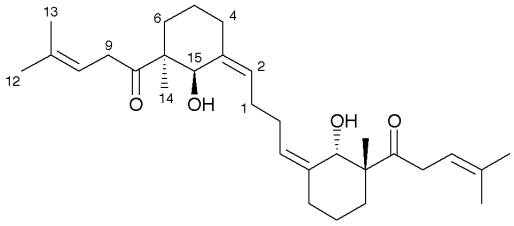
be a close isomer of abudinol (**56**), renamed abudinol A, where the ring E is also an oxepane, rather than a tetrahydropyran as in abudinol (**56**). This was confirmed by micro-acetylation of **55** affording a diacetate whereas abudinol A, possessing one secondary and one tertiary hydroxy, gave only a monoacetate.

The last, tetracyclic compound designated muzitone (**59**) has two hydroxytrimethyl oxepane systems, rings A and D. The ring A expanded by an additional cyclohexane ring B (C-6–C-11), and closing the fourth, a cycloundecanedione ring C completing the gross structure of compound **59**. Its stereochemistry was suggested on the basis of NOEs data in comparison with the corresponding data in abudinols and related compounds.

3.1.3 Compounds with a butylene bridge. The last group is constituted by a quite rare kind of triterpenoids. The unusual partially cyclised squalene skeleton present in these compounds had been reported until now in only one other non polyether natural product from a marine source, limatulone (**60**), a defensive allomone from the limpet *Collisella limatula*.[52]

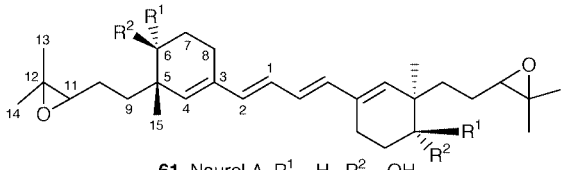


59 Muzitone

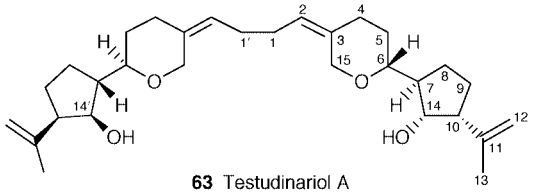


60 Limatulone

Thus, firstly two new stereoisomeric triterpene alcohols, naurols A (**61**) and B (**62**),[53] were isolated from a mixture of



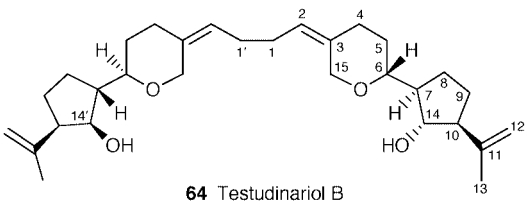
61 Naurol A $R^1 = H$, $R^2 = OH$
62 Naurol B $R^1 = OH$, $R^2 = H$



63 Testudinariol A

sponge specimens of *Rhaphisia* sp., order Poecilosclerida, family Tedaniidae, and another sponge classified as belonging to the order Axinellida, family Euryponidae, collected at Nauru Is. Hence the exact origin of the new products is unknown. Their structures possess an uncommon symmetrical carbon skeleton centred about a linear conjugated tetraene moiety and have only two carbocyclic rings. The structures were determined primarily from spectroscopic data revealing that the two compounds had near identical structures and confirmed that the two compounds had the same overall skeleton and double bond geometry and likely differed only in stereochemistry at the hydroxy carbon. Both alcohols are mildly toxic to murine lymphocytic leukaemia cells P-388, ED_{50} 4.6 and 4.4 μg mL^{-1}, respectively.

Finally, two novel triterpenoids, testudinariol-A (**63**) and testudinariol-B (**64**), were isolated from the skin and the mucus of the mollusc *Pleurobranchus testudinarius*,[54] compound **63** being the main liposoluble component of the defensive mucous secretion. The structure of testudinariol-A (**63**) has been assigned on the basis of extensive spectral studies. Its absolute configuration at the hydroxy carbon centre C-14 was deter-



64 Testudinariol B

mined by applying modified Mosher's methods consisting of submitting the product to esterification with (*R*) and (*S*)-MTPA chlorides. Analysis of the ^{1}H-NMR spectra of the two MTPA esters showed that the chirality of C-14(C-14′) was *S*. The structural work on the minor co-occurring metabolite, testudinariol-B (**64**) led to the epimer of **63** at C-10. The presence of **63** in the most exposed part of the animal and in the mucus is strongly indicative that this compound could act as a defensive allomone of *Pleurobranchus testudinarius*. Furthermore, testudinariol A (**63**) was ichthyotoxic in the test against *Gambusia affinis* at the concentration of 10 ppm.

3.2 Biogenetic considerations

The first group of these triterpenes comprises two separate cyclic systems obtained assumably by two separate cyclisations from triepoxysqualenes (**65**) in sipholanes and some sodwanones, or tetraepoxysqualenes (**66**) in most compounds. Each cyclisation is suggested to lead to the formation of a carbonium ion obtained from protonation of either an epoxide or one of the squalene double bonds (Scheme 4).

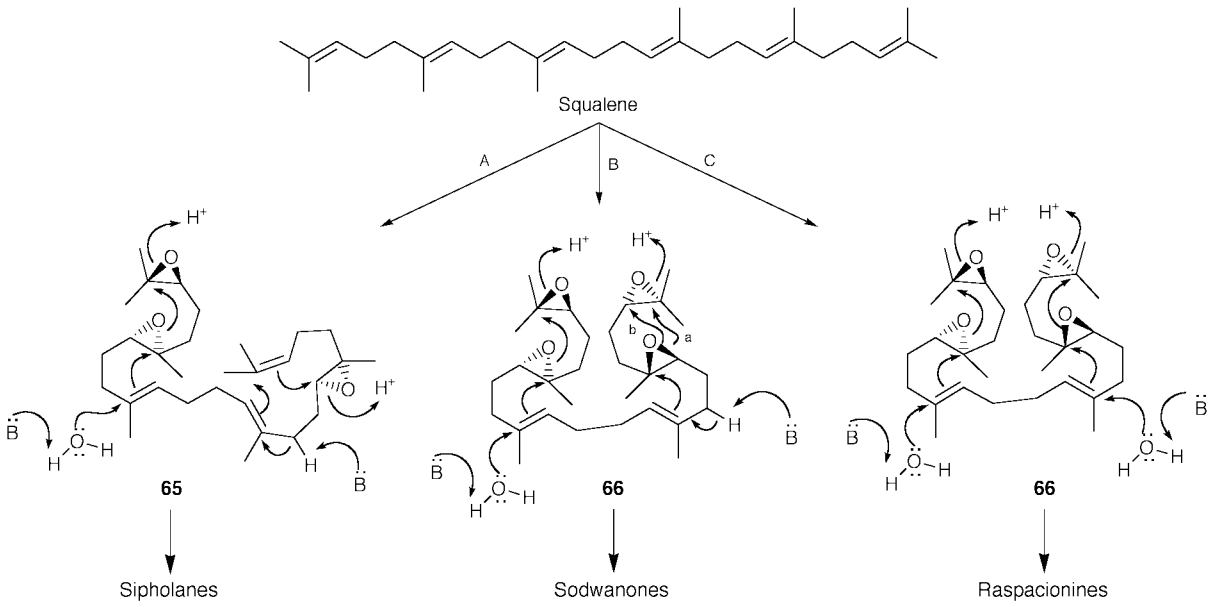
It appears that for the metabolites with two *trans*-perhydrobenzooxepine systems, as in the case of raspacionin (**32**) and related compounds, the common precursor could be (2,3*S*:6*S*,7*S*:18*S*,19*S*:22*S*,23)-tetraepoxysqualene (**66**) through two equivalent cyclisation patterns. Each process would consist of the protonation of the terminal epoxide (2,3 or 22,23), cyclisation and hydroxylation of one of the central double bonds (10–11 or 14–15, respectively) (Scheme 4). Raspacionin A (**33**) and sodwanone F (**46**) may subsequently undergo a 1,2-shift of the methyl group C-1 to C-11 induced for the formation of an oxygen bridge between C-4 and C-1, leading to a rearranged triterpenoid. Similarly, a single methyl migration takes place in the formation of the sodwanone M (**48**).

The precursor suggested for the biogenesis of the sipholanes is (2,3*S*:6*S*,7*S*:18*S*,19*S*)-triepoxysqualene (**65**). A cyclisation in an equivalent manner to that of the above metabolites would involve the (2,3*S*:6*S*,7*S*)-diepoxide fragment to yield the characteristic *trans*-perhydrobenzooxepine half of these metabolites. On the other hand, the octahydroazulene system would be generated by the 14,15-double bond that promotes a cyclisation to the terminal double bond and attacks the 18–19 protonated epoxide.

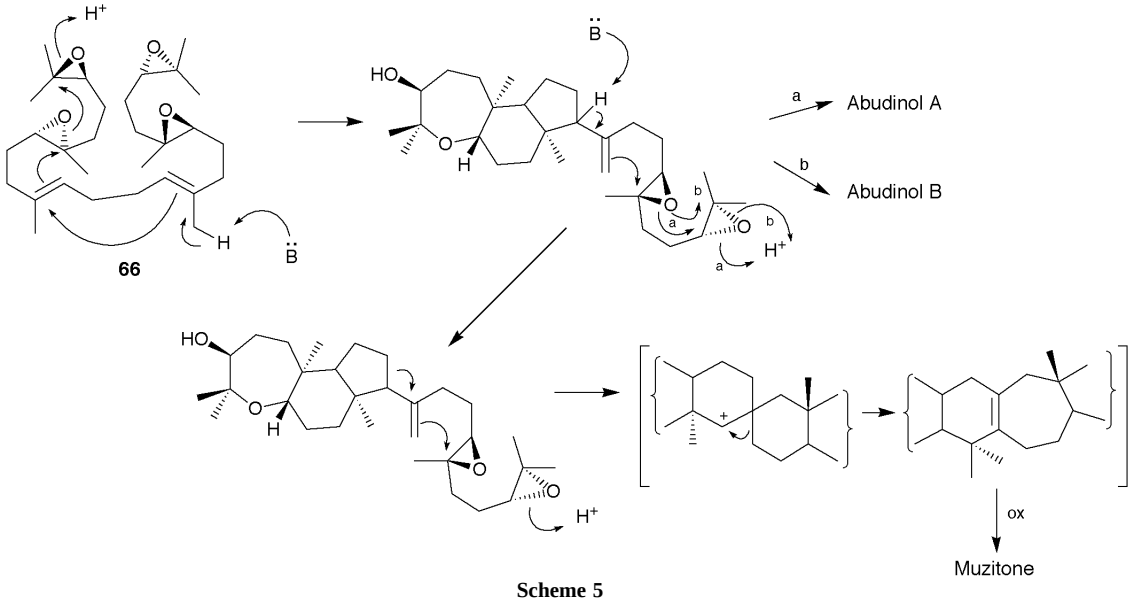
The dioxabicyclo[2.2.2]octane system present in sodwanones L (**49**), N (**52**), O (**53**) and P (**54**) would have been generated by cyclisation of the second half of the squalene precursor (**66**), as in route B.b in Scheme 4.

Of special interest to this group has been the isolation of sodwanones I (**51**) and N (**52**) showing partially cyclised systems indicating that the suggested classic biogenesis of the above metabolites involving two consecutive cyclisations, one for each separate system, may not be concerted.

A biogenesis has been suggested by Rudi *et al.*[51] for abudinols A (**56**), B (**58**) and muzitone (**59**). It is possible that the precursor tetrahydroxysqualene (**66**) can undergo a cyclisation after the functionalisation of Me-28 and explain the disappearance of the eight methyl groups in the abudinols and muzitone. Routes a and b (Scheme 5) are alternatives for the formation of either a THP or an oxepane ring, leading to abudinols A (**56**) and B (**58**), respectively. It is further suggested that an intermediate spiro compound is the precursor to explain the carbonyl groups at C-25 and C-14 in the structure proposed for muzitone (**59**).



Scheme 4



Scheme 5

No biogenetic interpretation of the formation of the last group has been put forward. It has just been mentioned that it would involve an unusual partial cyclisation of the squalene precursor.

Finally, from the studies summarised in this review, it seems clear that marine polyether triterpenoids have an important pharmacological potential. These compounds are unique in their structures and strong biological activities, and these properties convert them into interesting and useful tools in biomedical research implying cyclisation on to methyl-bearing carbons.

4 Acknowledgements

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