The Chemistry of Marine Pulmonate Gastropods

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Abstract. Secondary metabolites from pulmonate molluscs of the genera *Siphonaria*, *Onchidium,* and *Trimusculus* are described. *Siphonaria* and *Onchidium* biosynthesize mostly propionate-based metabolites whereas *Trimusculus* yields diterpene derivatives with a single type of labdane skeleton. The 42 regular polypropionates reported to date from *Siphonaria* are divided into two classes (class I, class II), based on their observed structural and stereochemical analogy. The strong resemblance between class I and cephalaspidean metabolites and between class II and onchidiid metabolites as well as the structural features of *Trimusculus*, in relation to the other pulmonates, encourage speculation about their biosynthetic and phylogenetic relationship. Class I metabolites could be suitable material to evidence that type I PKS modules are perhaps used iteratively in their biosynthesis.

5.1 Introduction

The gastropods form by far the largest and most diverse class of molluscs, comprising more than half of all mollusc species. The traditional division of gastropods into three subclasses, Prosobranchia, Opisthobranchia, and Pulmonata, was followed by several conflicting phylogenetic hypotheses and taxonomic classification systems (for a review of earlier work, see Bieler 1992). In an analysis of gastropod phylogeny based on a set of morphological and ultra-structural characters, Ponder and Lindberg (1997) demonstrated the probable monophyly of the gastropods and divided them into two major groups: subclass Eogastropoda, comprising the Patellogastropoda, and the remaining gastropods included in subclass Orthogastropoda that comprises the superorders Neritopsina, Vetigastropoda, Caenogastropoda, Heterobranchia, and the orders Opisthobranchia and Pulmonata. Patellogastropods and vetigastropods are all marine, while the neritopsines and caenogastropods are mostly marine, including a few freshwater and terrestrial groups. This newer classification, although currently recognized, will take some time to be widely applied.

Marine gastropods are the most diverse group of marine invertebrates. Within the gastropods, the subclass Pulmonata comprises marine groups, but the majority are freshwater or terrestrial. The marine members comprise six families in three orders, including air-breathing limpets (Siphonariidae, Trimusculidae), the mangrove onchidiid slugs

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Progress in Molecular and Subcellular Biology
Subseries Marine Molecular Biotechnology
G. Cimino, M. Gavagnin (Eds.): Molluscs

(Onchidiidae), the estuarine ear shells (Ellobiidae), and mud snails (Amphibolidae). The pulmonates have no gills, but feature a vascularized mantle cavity which serves as a functional lung sac for air-breathing both above and under water, hence the name "pulmonates".

This chapter deals with the chemistry of marine pulmonates belonging to the families Siphonariidae, Onchidiidae (Order Systellomatophora), and Trimusculidae (Order Basommatophora).

5.2 Secondary Metabolites from *Siphonaria*

The Siphonariidae are regarded as the most primitive of pulmonates which probably have a marine ancestry and may represent an evolutionary link between marine and terrestrial gastropods (Hyman 1967; Purchon 1979). Nearly all siphonariids are unpalatable to predators since, when disturbed by a potential predator, they secrete a white mucus from lateral epidermal glands that contain polypropionate metabolites (Davies-Coleman and Garson 1998). The Siphonariidae are a diverse family, with over 60 recorded species; and their phylogeny is still in debate, even the number of genera being contentious. While only two genera, *Siphonaria* and *Williamia*, were previously recognized with two subgenera: *Liriola* and *Siphonaria*, other authors have since recognized four genera, *Siphonaria*, *Williamia*, *Kerguelenella*, and *Benhamina* (Hodgson 1999).

Of the marine pulmonates, the genus *Siphonaria* has been the most studied. The siphonariids are characterized by their ability to synthesize polypropionate metabolites with different types of skeletons, approximately one-third of which are acyclic while the remainder contain a 2-pyrone, 4-pyrone, or furanone ring. A defensive value of the polypropionates is not incompatible with the fact that *Siphonaria* spp. are physically protected by a shell (Faulkner 1992; Cimino and Ghiselin 1999). The polyketides isolated from *S. diemenensis* represented the first polypropionates obtained from the pulmonates since, until then in marine invertebrates, polypropionate metabolites had been isolated from sacoglossans (Ireland and Faulkner 1981).

Within the metabolites from *Siphonaria*, structural and stereochemical analogies are observed at specific positions. These analogies are used as criteria to assign the compounds from *Siphonaria* to two classes: class I comprises those metabolites that possess an identical configuration at all the comparable stereochemical centers, class II comprises those metabolites that possess an identical configuration at only a part of the comparable stereochemical centers of a complex pattern of functional groups. The compounds included in this class exhibit analogies with the model of

Celmer (1965), suggesting that the producer organism adopts a similar biosynthetic pattern.

5.2.1 Class I Siphonariid Polypropionates

Class I comprises approximately half of the polypropionate metabolites isolated from *Siphonaria* and includes essentially acyclic compounds, compounds with a 2-pyrone ring and those having a furanone ring (Fig. 5.1). Class I compounds have the following structural and stereochemical characteristics:

– The saturated linear alkyl chain is made up of at least three propionate units

– All the methyl groups of this portion of the linear chain have an *S* configuration

– The 2-pyrone ring and the furanone ring are characteristic of class I

The secondary metabolites comprised in class I have been isolated, as a whole, from seven species of the genus *Siphonaria*: *S. diemenensis*, *S. pectinata*, *S. grisea*, *S. virgulata*, *S. lessoni*, *S. capensis*, and *S. concina*, noting that no class II metabolite has been characterized from these species. Since many of these metabolites have been isolated from different species along the years, Table 5.1 summarizes class I siponariid polypropionates with attention to their species origin, locality, and bioactivity data when available.

The first polypropionates from *Siphonaria*, diemenensins A (**1**) and B (**2**), were isolated from *S. diemenensis* collected off the southeast coast of Australia. The identification of methyl (2*S*,4*S*)-2,4-dimethylheptanoate from the chemical degradation of **1** allowed its absolute configuration to be established (Hochlowski and Faulkner 1983).

Pectinatone (**3**) was isolated from the skin extract of *S. pectinata* (Florida). The absolute stereochemistry of **3** was initially determined by comparison of the value of the optical rotation of the methyl ester **4**, obtained by ozonolysis of **3** and subsequent methylation, with that described in the literature for methyl (2*S*,4*R*,6*S*)-2,4,6-trimethylnonanoate (Biskupiak and Ireland 1983). However, the true configuration of C-11 was resolved by X-ray diffraction analysis of **3** obtained from both *S. virgulata* (Garson et al. 1990) and *S. grisea* (Norte et al. 1990). The total synthesis of (+)-pectinatone confirmed the structure indicated in **3** (Birkbeck and Enders 1998).

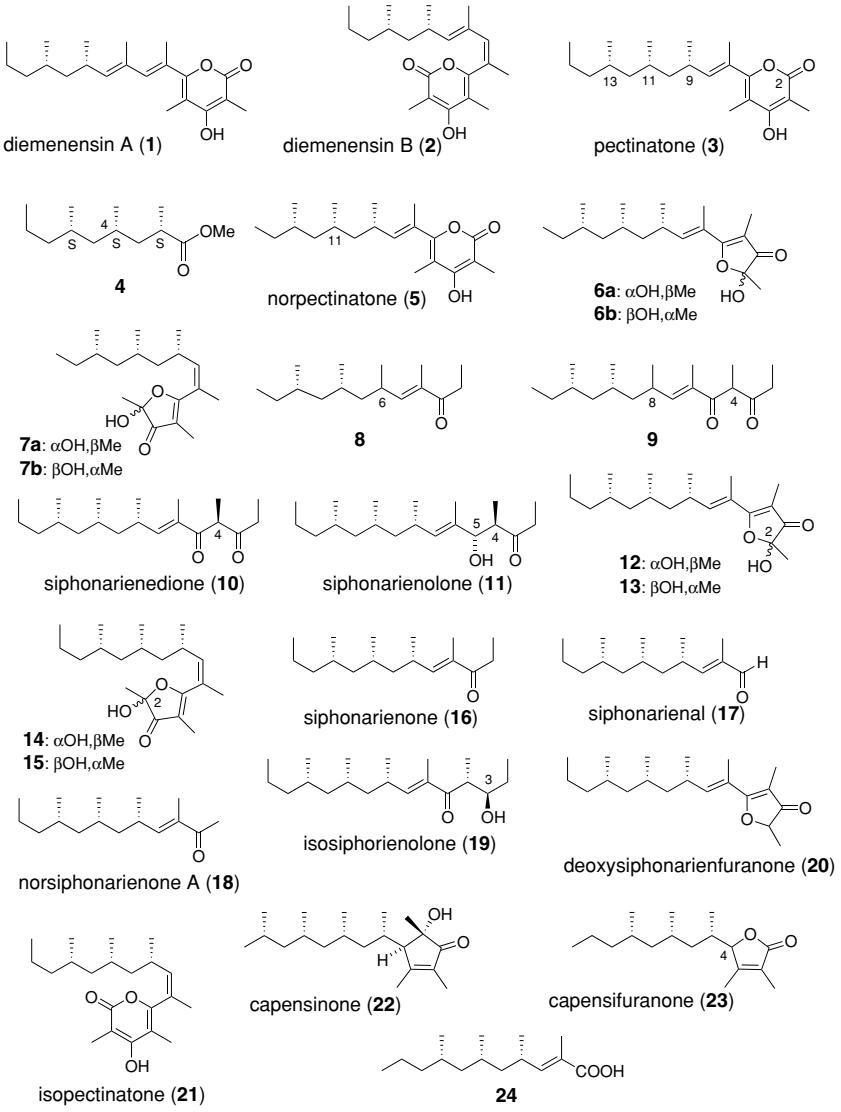


Fig. 5.1. Class I siphonariid polypropionates

Table 5.1. Class I siphonariid polypropionates

species	location	compounds	bioactivity	references
S. diemenensis	SE Australia	diemenensin A (**1**)	antimicrobial	Hochlowski and Faulkner (1983)
		diemenensin B (**2**)	cell division inhibition	
		6a, 6b		Capon and Faulkner (1984)
S. pectinata	Florida	pectinatone (**3**)	antimicrobial	Biskupiak and Ireland (1983)
	Cádiz, Spain	**3**	cytotoxic	Paul et al. (1997)
		siphonarienedione (**10**)		
		siphonarienolone (**11**)	cytotoxic	
		12, 13, 14, 15	cytotoxic	
		siphonarienone (**16**)		
		norsiphonarienone A (**18**)		
		isosiphorienolone (**19**)		
		deoxysiphonarienfuranone (**20**)		
		isopectinatone (**21**)		
S. lessoni	Chile	norpectinatone (**5**)		Capon and Faulkner (1984)
		6a, 6b, 8, 9		Rovirosa et al. (1991)
S. grisea	Canary Islands	**3, 10, 11, 12, 13, 14, 15, 16**	antimicrobial	Norte et al. (1988, 1990)
	Dakar, Senegal	**3, 10, 11, 12, 13, 16**		Norte et al. (1994)
		siphonarienal (**17**)		
S. capensis	South Africa	**12, 13, 14, 15**		Beukes and Davies-Coleman (1999)
		capensinone (**22**)		
		capensifuranone (**23**)		
		24		
S. virgulata		**3, 5**		Garson et al. (1990)
S. concina		**3**		Davies-Coleman and Garson (1998)

Norpectinatone (**5**) was isolated from *S. lessoni* (Chile). This same extract afforded the 1:1 mixture of furanones **6a** and **6b** (Capon and Faulkner 1984). The 11*R* stereochemistry, originally proposed by comparison of the optical rotation of the methyl ester, obtained by degradation of compound **5**, with that of four isomers of 2,4,6-trimethylnonanoate, was questioned by Oppolzer et al. (1986) since the spectroscopical data of norpectinatone, obtained by means of enantioselective synthesis, did not coincide with those of the natural product. Subsequently, X-ray diffraction analysis of pectinatone indicated that the configuration at C-11 of norpectinatone should be revised to 11*S* as indicated in **5** (Garson et al. 1990). The absolute stereochemistry initially proposed for the linear chain of the furanones **6a** and **6b** was revised to all *S* stereoisomers (Rovirosa et al. 1991). Moreover, the authors proposed that the mixture of furanones is formed by the epimers at C-2 and not by the geometric isomers *E* and *Z*, although it is possible that, on standing, **6a** and **6b** isomerize to **7a** and **7b** as occurred with the mixture of compounds **12** and **13** (Norte et al. 1990).

Compounds **8** and **9** were isolated from *S. lessoni* (Chile). The configuration of C-6 in **8** and those of C-4 and C-8 in **9** remain undetermined (Rovirosa et al. 1991). However, the stereochemical regularity observed in the methyl groups of the polypropionic chain of the group of metabolites comprising class I allows the prediction of an *S* configuration for Me-6 and Me-8 in **8** and **9**, respectively.

The acyclic polypropionates siphonarienedione (**10**) and siphonarienolone (**11**) were isolated from *S. grisea* (Canary Islands). Oxidative degradation of **10** and **11** gave rise in both cases to the same 2,4,6-trimethylnonanoic acid, whose optical rotations indicated that they were in the same enantiomeric series as (2*S*,4*S*,6*S*)-trimethylnonanoic acid. However, the stereochemistry at C-4 and C-5 of **11**, assigned on the basis of the comparison of their spectral data with those of compound **29**, was incorrect (Norte et al. 1988), as was later verified by total synthesis of both natural compounds, establishing the correct configurations as those represented in **10** and **11** (Calter and Liao 2002; Magnin-Lachaux et al. 2004).

From another study of *S. grisea*, the following metabolites were obtained: siphonarienfuranones **12** and **13** as an inseparable mixture of epimers at C-2, the epimeric mixture of **14** and **15**, as well as siphonarienone (**16**). The absolute stereochemistry of the side-chain of **12** and **13** was established by chemical degradation (Norte et al. 1990). The enantioselective synthesis of (+)siphonarinenone (**16**) has been reported (Abiko and Masamune 1996).

S. grisea (Dakar) yielded siphonarienal (**17**). The absolute configuration was established by means of enantioselective synthesis (Norte et al. 1994).

The novel polypropionates **18–21** were isolated from *S. pectinata* (Cádiz, Spain; Paul et al. 1997). The absolute stereochemistry of **18** was proposed on the basis of biogenetic considerations. The configuration at

C-3 of **19** was established by comparison with the spectroscopical data of siphonarienolone (**11**).

S. capensis, endemic to South Africa, afforded the novel metabolites capensinone (**22**), capensifuranone (**23**), and (2*E*,4*S*,6*S*,8*S*)-2,4,6,8-tetramethyl-2-undecanoic acid **24**; (Beukes and Davies-Coleman 1999). The configuration of the side-chain chiral centers of **22** and **23** was assumed to be *S* on the basis of biogenetic considerations, the configuration of C-4 in **23** remaining undetermined. The oxidative degradation of **24** to (2*S*,4*S*,6*S*)-2,4,6 trimethylnonanoic acid allowed its absolute configuration to be established.

5.2.2 Structural Analogy Between Class I and Cephalaspidean Polypropionates

The siphonariid metabolites from this class possess an alkenyl chain that often contains an α-pyrone or a furanone ring. Remarkably, within the group, they share the same absolute configuration at all comparable stereocenters. It is interesting to observe that they are similar to propionates from *Bulla striata*, *B. gouldiana*, *B. speciosa*, *Aglaja depicta*, and *Navanax inermis* cephalaspidean molluscs (Cimino and Sodano 1993). Examples are shown in Fig. 5.2 in which representative linear alkenyl chain, alkenyl-furanone, and alkenyl-2-pyrone metabolites are compared. The comparison of class I siphonariids and cephalaspidean metabolites suggests that an olefin reductive enzymatic process occurred at certain positions along the chain to give all *S* stereocenters. However, this reductive process does not occur at the olefinic terminus of the linear chain (Fig. 5.2, dashed boxes). Aglajne-1 and aglajne-3 incorporate [1-^{14}C]-propionate when the animals are supplemented with the sodium salt of the precursor, evidencing that these compounds derive de novo from propionate (Fontana et al. 2004).

Polypropionates are biosynthesized by polyfunctional type-I polyketide synthases (PKSs), also called modular PKSs. These enzyme complexes use a wide range of organic acids as starter units; and the extenders are generally malonyl and methylmalonyl units. Polyketides are assembled by sequential decarboxylative condensation of short carboxylic acids and the PKSs contain a module for every cycle of chain extension; and this correlation is termed colinearity. In modular PKSs, typified by 6-deoxyerythronolide synthesis (Cortés et al. 1990; Donadio et al. 1991), each module contains the requisite enzymatic domains: ketosynthase (KS) catalyzing the chain elongation, acyl transferase (AT) selecting the extender and acyl carrier protein (ACP) mediating the correct transfer of the growing polyketide chain. These domains are covalently linked in the order: KS – AT – reduction domain loop – ACP. The three main reductive

domain loops found in modules are: ketoreductase (KR), dehydratase (DH)-KR or DH-enoyl reductase (ER)-KR domains which determine, by a processive mechanism, the extent of β-ketone group processing in each cycle. Domain exchange experiments have shown that KR domains are responsible for determining the final stereochemistry at chiral centers derived from reduction of β-ketone to alcohols, but it is still unclear exactly how modular PKSs control alcohol stereochemistry in the nascent oligoketide chain. Predictive methods suggest that the DH domains of modular PKSs normally act on (3*R*)-hydroxyacyl chains to give *trans*-double bonds. DH and ER domains must determine alkyl stereochemistry when incorporation of a branched extender is followed by complete reduction of the β-ketone (Staunton and Weissman 2001).

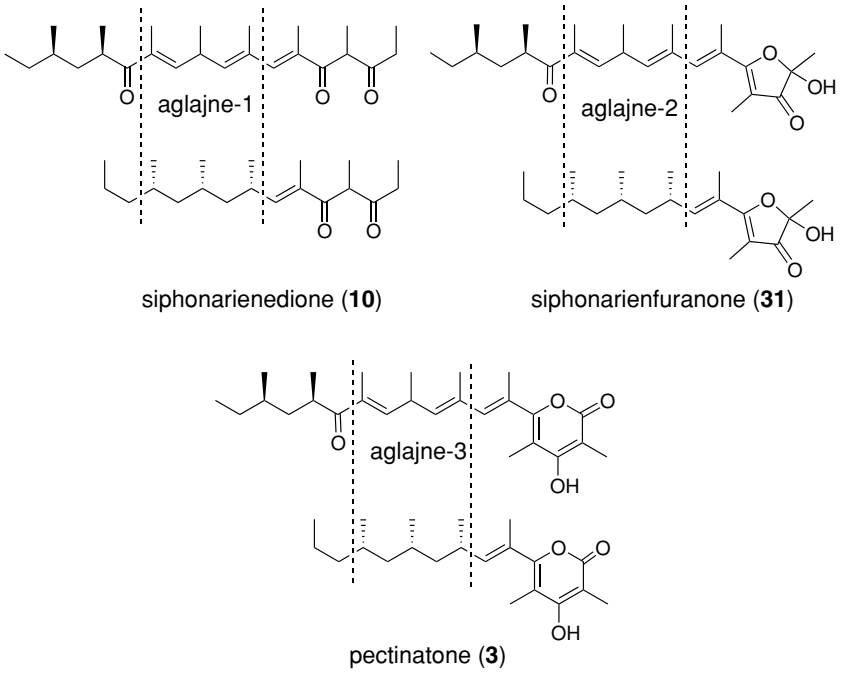


Fig. 5.2. Structural similarities between class I and cephalaspidean polypropionates

The striking properties of class I metabolites encourage speculation about whether the putative reductive loop leading to complete reduction of the β-ketone of the extender methylmalonate to methylene is repeated over and over again to elongate the chain. This would account for the secondary methyl groups at alternating positions and raises the question of whether, as occurs with bacterial type I, the PKSs include three

modules, each containing the three core domains that are essential for formation of each of the C–C bonds, or whether there is a type I system in which iterative rounds of chain extension occur as a programmed event by using one module three times. Indeed, it has been proposed that the biosynthesis of borrelidin involves an iterative use of module 5 (BorA5) of its biosynthetic gene cluster (Olano et al. 2004). Because there is a 1,3, 5-trimethylhexyl moiety identity between borrelidin and class I metabolites (Fig. 5.3), this latter organization seems likely, therefore, for class I siphonariid metabolite biosynthesis.

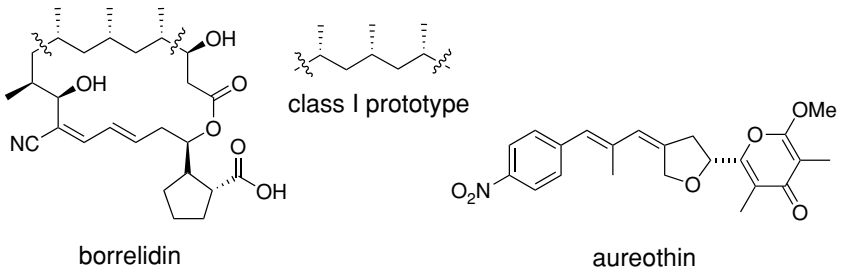


Fig. 5.3. Conserved configuration in the respective 1,3,5-trimethylhexyl moiety of Class I and borrelidin. Structure of aureothin

In the genesis of the cephalaspidean aglajne-1 to aglajne-3, the extent of the reductive carbonyl unit process in a comparable chain-extension cycle has been truncated twice by loss of the enoyl reductase domain, thus generating a double bond in the chain-extension product (Fig. 5.2). Although the generated couple of double bonds are located at nonalternating positions (one cycle is fully reduced), the possibility still exists of an iterative type I PKS by using a module twice, similar to module 1 (AurA1) of the biosynthetic gene cluster responsible for aureothin biosynthesis (He and Hertweck 2003), by considering the two alternate linear double bonds. Class I metabolites could thus be a source of suitable material to evidence that type I PKS modules are perhaps used iteratively in their biosynthesis.

It is unclear exactly how modular PKSs control methyl stereochemistry in the growing chain and the enoyl reductase domains occur relatively infrequently in PKS modules, being the least studied of all of the constituent domains. Because of this, the extended network analogy of the respective pairs: aglajne-1/siphonarienedione, aglajne-2/siponarienfuranone and aglajne-3/pectinatone (Fig. 5.2) and the fact that class I and cephalaspidean metabolites provide examples of modification of the level of reduction of the growing chain, it seemed that the tandem compounds could be good models for the cloning of the biosynthetic gene cluster of the producers. This might yield insights into our understanding of the molecular mechanistic basis of the enzymatic

stereocontrol for determining the final stereochemistry at chiral centers derived from the reduction of β-ketone in the growing chain of the polypropionate biosynthesis. Also, the possibility to virtually convert syphonariids into producers of metabolites characteristic of cephalaspidean (and vice versa) by entire module exchange experiments in their respective PKSs, appears to constitute an exciting challenge. The core analogy of those pairs of compounds may preserve intact the acyl carrier protein–ketosynthase (ACP–KS) bi-domain that spans the junction between successive modules, conferring an advantage on any eventual goal undertaken in this sense (Gokhale et al. 1999; Ranganathan et al. 1999).

Although the aforesaid discussion on the class I/cephalaspidean polypropionates is speculative and there is no evidence to prove this biosynthesis, the rationalization of the observed structural and stereochemical analogies between these compounds may be useful in future related research.

5.2.3 Class II Siphonariid Polypropionates

Whilst class I secondary metabolites are structurally mundane in that almost the only real variation is in the length of the alkyl chain, a feature of class II is that it yields a profuse polyoxygenated network that frequently cyclizes to spiroacetal and/or γ-pyrone rings; and these functionalities are not observed in class I compounds. The striking structural and stereochemical correlation at comparable stereocenters between members of macrolide classes of actinomycete antibiotics and siphonariid metabolites of class II suggests that these compounds may share a common genetic origin (Garson et al. 1994b). The class II polypropionates (Fig. 5.4) have been isolated from the following ten species: *S. denticulata*, *S. australis*, *S. zelandica*, *S. normalis*, *S. lacinosa*, *S. baconi*, *S. atra*, *S. maura*, *S. funiculata* and *S. serrata*. None of the following described metabolites of this class has been found in class I. Table 5.2 summarizes class II siphonariid polypropionates with attention to their species origin, locality and bioactivity data when available.

The epimers denticulatins A (**25**) and B (**26**) were isolated from *S. denticulata* (Australia). The structure and relative stereochemistry of **26** was established by X-ray diffraction analysis and its absolute configuration was deduced on the basis that the levorotatory enantiomer **27**, obtained by degradation of both compounds, has an *R* configuration at C-4 (Hochlowski et al. 1983). A number of stereocontrolled syntheses of denticulatin A and denticulatin B have been reported; and pure samples of synthetic denticulatins A and B were found to interconvert on silica gel, indicating that the natural products from *S. denticulata* may actually be only a single compound which isomerizes at C-10 on chromatographic

isolation (Ziegler and Becker 1990; Andersen et al. 1991a,b; Paterson and Perkins 1992, 1996; Oppolzer et al. 1995; De Brabander and Oppolzer 1997). Biosynthetic studies show that the denticulatins originate in the condensation of propionate units and not in the methylation of a standard polyacetate chain (Manker et al. 1988).

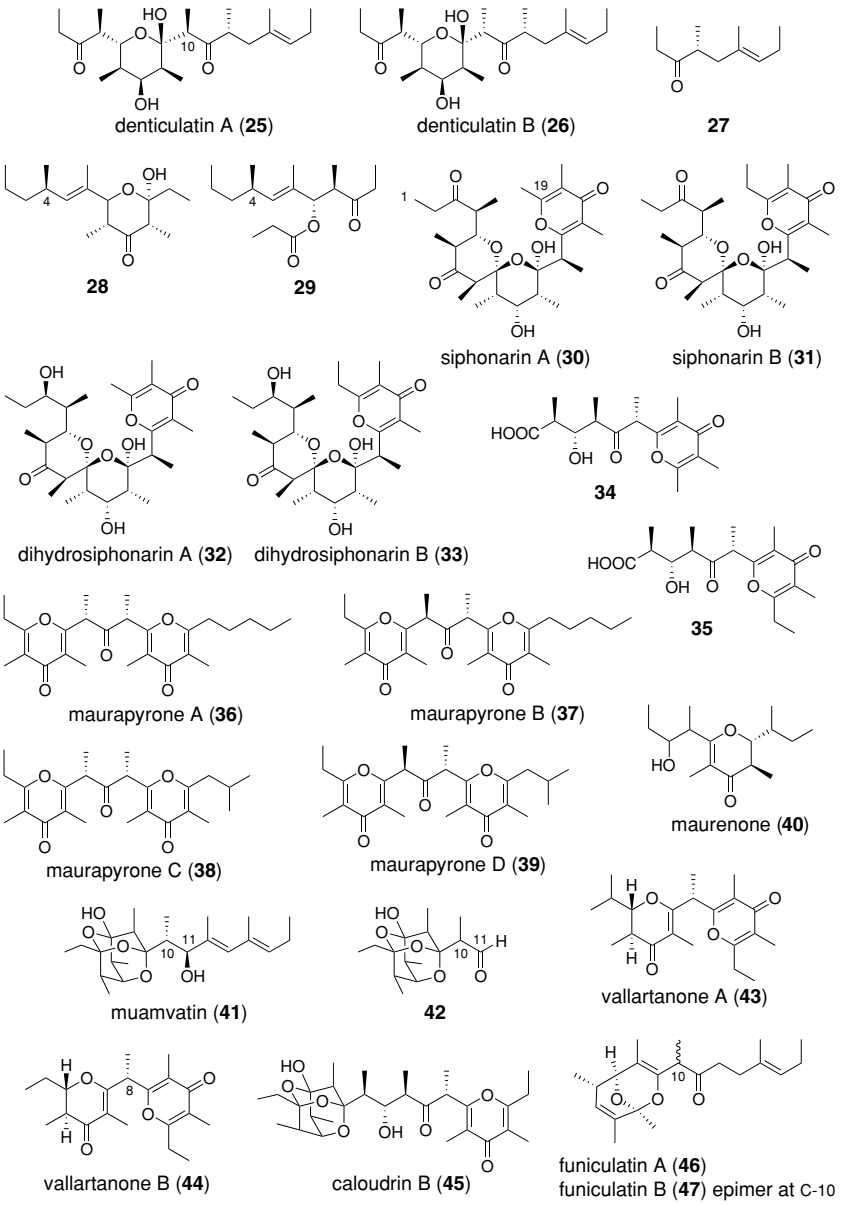


Fig. 5.4. Class II siphonariid polypropionates

Table 5.2. Class II siphonariid polypropionates

species	location	compounds	bioactivity	references
S. denticulata	New South Wales (Australia)	denticulatin A (**25**) denticulatin B (**26**)	ichthyotoxicity	Hochlowski et al. (1983)
S. australis	Auckland (New Zealand)	**28, 29**		Hochlowski and Faulkner (1984)
S. zelandica	Sydney (Australia)	siphonarin A (**30**) siphonarin B (**31**)		Hochlowski et al. (1984)
	Queensland (Australia)	caloudrin B (**45**)		Blanchfield et al. (1994)
S. atra	Townsville (Australia)	**30, 31**		Hochlowski et al. (1984)
S. baconi	Melbourne (Australia)	**30** baconipyrone A (**48**) baconipyrone B (**49**) baconipyrone C (**50**) baconipyrone D (**51**)		Manker et al. (1989)
S. normalis	Oahu (Hawaii)	dihydrosiphonarin A (**32**) dihydrosiphonarin B (**33**)		Hochlowski et al. (1984)
	Maumatu (Fiji)	maumvatin (**41**)	non-antimicrobial	Roll et al. (1986)
S. lacinosa	Townsville (Australia)	**32, 33**		Hochlowski et al. (1984)
S. maura	Jaco Beach (Costa Rica)	maurapyrone A (**36**) maurapyrone B (**37**) maurapyrone C (**38**) maurapyrone D (**39**) maurenone (**40**)	antibacterial	Manker et al. (1986)
	Puerto Vallarta (Mexico)	vallartone A (**43**) vallartone B (**44**)	larval settlement inducer antifeeding	Manker and Faulkner (1989)
S. funiculata	Queensland (Australia)	funiculatin A (**46**) funiculatin B (**47**)		Blanchfield et al. (1994)
S. serrata	South Africa	siserrone A (**52**)		Brecknell et al. (2000)

Compounds **28** and **29** were isolated from *S. australis* (New Zealand; Hochlowski and Faulkner 1984). The synthesis of the linear compound allowed the establishment of its relative stereochemistry; and the absolute configuration, represented in **29**, was subsequently determined by circular dichroism. Since **29** can be derived from **28** by a retro-Claisen condensation process, both compounds possess the same stereochemistry and absolute configuration 4*R*, 7*S*, 8*R* (Sundram and Albizati 1992).

Siphonarins A (**30**) and B (**31**) were isolated from a mixed extract (~1:4) of *S. zelandica* and *S. denticulata* collected in Australia; and it was later shown that both compounds were present in the extract of *S. zelandica* (Hochlowski et al. 1984). The relative stereochemistry of **30** was established by X-ray diffraction analysis.

From *S. normalis* (Hawaii) and *S. lacinosa* (Australia) the dihydrosiphonarins A (**32**) and B (**33**) were obtained, together with the degradation products **34** and **35** (Hochlowski et al. 1984).

The absolute configuration of siphonarin A (**30**) was established based on an X-ray study of its *p*-bromophenyl boronate derivative. The methyls of the tetrahydropyrone ring of **30** have the same configuration as those in the tetrahydropyrone ring of **25** and **26** (Garson et al. 1994a,b). In contrast, the synthesis of the enantiomer of **35** shows that it is opposite to that obtained from the degradation of **33** and confirms the absolute stereochemistry of the siphonarins (Paterson and Franklin 1994). Biosynthetic experiments indicate a preference for an acetate chain starter unit in the biosynthesis of **30** and define the direction of chain assembly as from C-19 to C-1, demonstrating the presence of a functioning methylmalonyl-CoA mutase in *S. zelandica* (Garson et al. 1994a). The total synthesis of **31** and **33** has been achieved (Paterson et al. 2002).

Two pairs of racemic diastereoisomers maurapyrones A–D (**36–39**), together with maurenone (**40**), were isolated from *S. maura* (Costa Rica). The relative stereochemistry of maurapyrone A was established by X-ray diffraction analysis (Manker et al. 1986).

The triacetal muamvatin (**41**) containing an unusual 2,4,6-trioxaadamantane ring system was isolated from *S. normalis* (Fiji). Its side-chain stereochemistry could not be fully determined (Roll et al. 1986). Later, a stereocontrolled synthesis of both epimers at C-10 of **42** and the comparison of their optical rotation with that of the corresponding aldehyde obtained by degradation of **41** allowed an *R* configuration to be established at C-10 (Hoffmann and Dahmann 1993; Dahmann and Hoffmann 1994). On the basis of Mosher analysis of the full synthetic C-11 muamvatin epimer, the absolute stereochemistry represented in **41** was established. During the synthesis of **41**, it was observed that the trioxaadamantane system is produced in silica gel by rearrangement, suggesting that muamvatin may be an artifact produced during purification (Paterson and Perkins 1993).

Vallartanones A (**43**) and B (**44**) were isolated from *S. maura* (Mexico). The absolute configuration of **43** was established by circular dichroism (Manker and Faulkner 1989). However, the total synthesis of **44** and circular dichroism studies indicate that the configuration at C-8 should be revised to *S*, suggesting the same configuration for **43** (Arimoto et al. 1996a,b).

A new collection of *S. zelandica* (Australia) yielded caloudrin B (**45**), while *S. funiculata* afforded funiculatins A (**46**) and B (**47**), epimeric at C-10 (Blanchfield et al. 1994). The relative stereochemistry of the side-chain and the absolute stereochemistry were inferred from biosynthetic comparison with the above known polypropionates and by correlation of funiculatin A with denticulatin A (**25**). The stereochemistry at C-10 of funiculatin A could not be unambiguously determined.

5.2.4 Class II Polypropionates with a Noncontiguous Propionate Skeleton

The polypropionates listed below possess a skeleton whose propionate units are noncontiguous, as could be expected from regular polyketide biosynthesis (Fig. 5.5). Interestingly, naturally occurring metabolites belonging to this class, membrenones, have also been found in Notaspidea (Opisthobranchia), a related but taxonomically distant taxa (Ciavatta et al. 1993).

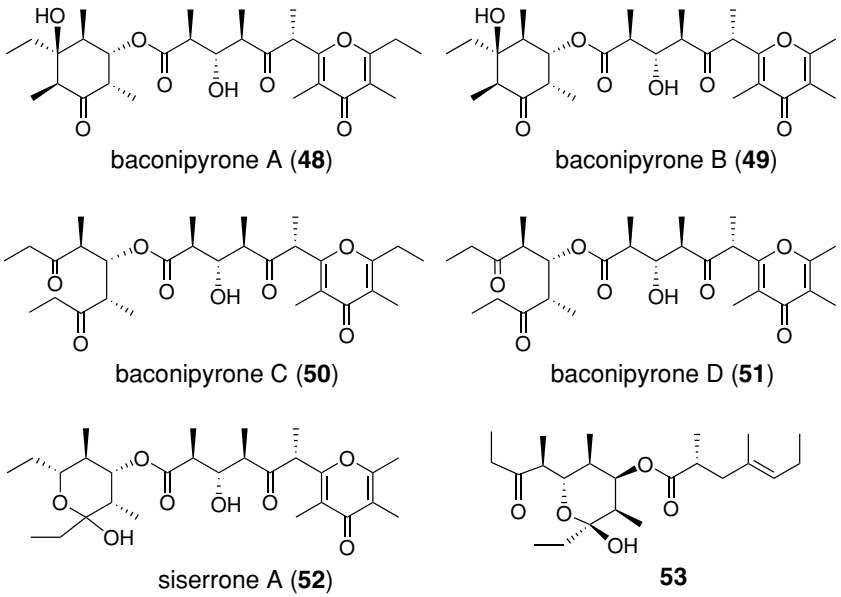


Fig. 5.5. Class II polypropionates with a noncontiguous propionate skeleton

Baconipyrones A–D (**48**–**51**) have been isolated from *S. baconi* (Australia). The structure of **49** was determined by X-ray diffraction studies. These compounds are presumed to be artifacts that derive from naturally occurring siphonarins (Manker et al. 1989). The enantioselective synthesis of **50** allowed the absolute configuration of the baconipyrones to be established and showed it to be in accord with that determined for the siphonarins (Paterson et al. 2000).

Specimens of *S. serrata* (South Africa) afforded siserrone A (**52**). The fact that the presence of baconipyrones was not detected in a sample of *S. baconi* from Sorrento (Australia) and that a second collection of *S. serrata* only gave **52** and no siphonarins leads to the conclusion that the siphonarins may be the precursors of these metabolites (Brecknell et al. 2000). However, it has been shown that base-catalyzed rearrangement of denticulatin A (**25**) yields the polypropionate ester **53** and funiculatin A (**46**). Those findings, together with the fact that denticulatins A and B undergo facile interconversion under mild conditions on silica gel (Paterson and Perkins 1992), appear to point to a non-natural origin for the polypropionate esters of siphonariids (Brecknell et al. 2000).

5.2.5 Structural Analogy Between Class II and Bacterial Metabolites

In 1965, Celmer noted that there are strong position-specific structural analogies between families of macrolides of various sizes. An intriguing feature, which emerges when compounds **25**, **30**, **36**, **41**, and **45** are compared with one another, is that they all share a common structural and stereochemical tetrapropionate unit, exemplified in the dashed box of the siphonarin A precursor, as shown in Fig. 5.6 (Garson et al. 1994b). This block is also present in the Cane–Celmer–Westley PAPA model (**56**) for polyethers, demonstrating that they all share a common fragment in their PKS products (Cane et al. 1983). The occurrence of common structural motifs in the bacterial macrolide, polyether antibiotics and siphonariid polypropionates first suggested they share a common biosynthetic origin, manifested in stereochemical control at the alkyl branching centers.

5.2.6 Siphonariid Nonpropionate-Derived Metabolites

A study of the composition of the fatty acids of *S. denticulata* (Queensland, Australia) allowed, in addition to the characterization of a high number of common fatty acids, the identification of two new acids (**54**, **55**; Fig. 5.7). Their structures were confirmed by total synthesis (Carballeira et al. 2001).

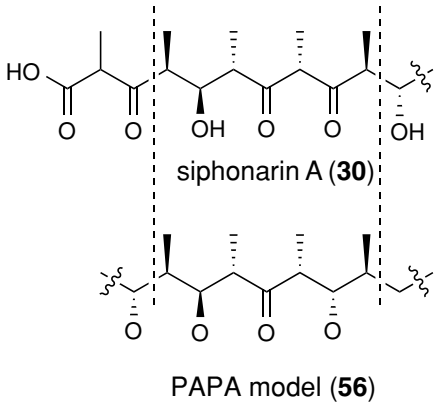
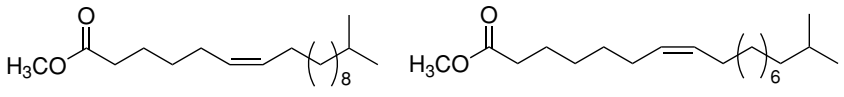


Fig. 5.6. Comparison of siphonarin open chain and PAPA model core



54 **55**

Fig. 5.7. Fatty acids from *Siphonaria*

5.3 Secondary Metabolites from *Onchidium*

The onchidioideans are shell-less marine molluscs. They are usually oval in shape with a dorsally arched notum bearing warts and papillae; and there are glands which apparently secrete noxious fluids at the sides of the body. They feed on the organic film of diatoms, other algae and bacteria which coat rocks and mud on sheltered intertidal shores.

Six species of the genus *Onchidium* have been studied to date. The greater part of the isolated compounds are isomeric polypropionates whose skeleton contains a γ-bispyrone ring and a great abundance of chiral centers (Fig. 5.8).

The first metabolite isolated from species of the genus *Onchidium* was the sesquiterpene onchidal (**57**; Ireland and Faulkner 1978). The isolation of an isoprenic derivative from this genus is unusual and noteworthy. This sesquiterpenic compound, presumed to play a defensive role, has also been isolated from *O. borealis* and *O. patelloides* (Manker and Faulkner 1987).

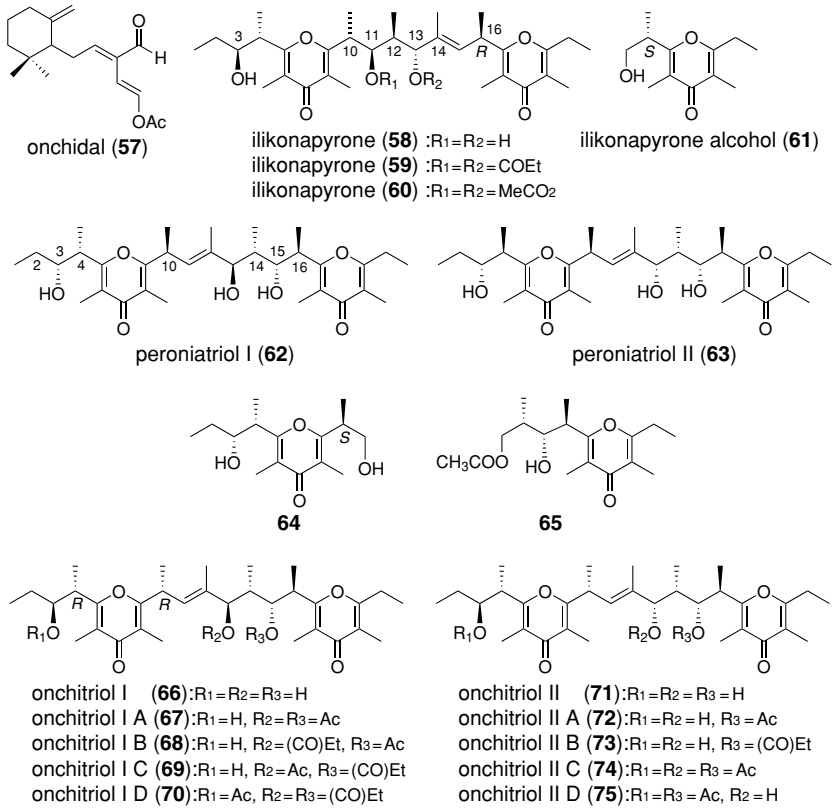


Fig. 5.8. Polypropionates from *Onchidium*

A mixture of esters based on the bispyrone alcohol ilikonapyrone (**58**) was isolated from *O. verruculatum* collected in Hawaii. Saponification of the mixture afforded the triol **58** whose structure, containing two γ-pyrones, was established by spectroscopical methods and chemical degradation. Its relative stereochemistry was established by X-ray analysis of the acetonide **60**. These compounds are considered defensive substances of Hawaiian *O. verruculatum*. The opposite signs of the optical rotations of fragment **61** obtained by: (a) oxidative degradation of **58** (Ireland et al. 1984) followed by reduction and (b) enantioselective synthesis (Arimoto et al. 1993) allowed the absolute configuration of **58** to be established.

Two cytotoxic metabolites, peroniatriols I (**62**) and II (**63**), have been isolated from the saponified extract of *Peronia peronii* (Guam; Biskupiak and Ireland 1985).

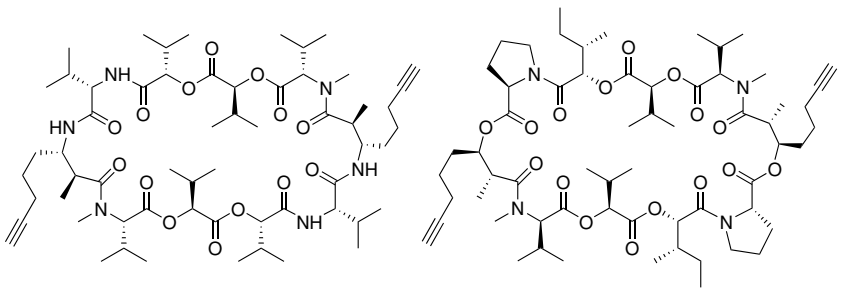
Comparison of the spectroscopical data and the optical activities of all the synthetic diastereoisomers of fragment **64** with those obtained from the natural products **62** and **63** allowed: (a) correction of the stereochemistry at

C-4 proposed for peroniatriol **62**, (b) confirmation of the 3*S* stereochemistry proposed for **62** and (c) proposal of the same configuration at C-10 for both compounds (Arimoto et al. 1990). The above-mentioned oxidative degradation of compounds **62** and **63** produced the same **65** fragment. Synthesis of the two enantiomeric forms of fragment **65** and comparison of the optical rotation of the synthetic fragments with those obtained from the natural products established the 14*R*, 15*R*, 16*R* configuration for both compounds.

In contrast, comparison of the spectral data and the optical rotation of the synthetic fragment **64** with an *S* configuration at the position equivalent to C-10 of peroniatriols I and II with the **64** fragment obtained from **62** confirmed the *S* configuration at C-10 for compounds **62** and **63** (Arimoto et al. 1993).

Eight new esters were isolated from an *Onchidium* sp. collected in New Caledonia. Four of them are esters of onchitriol I (**66**), onchitriols I A–D (**67**–**70**), and the remaining four are from onchitriol II (**71**), onchitriols II A–D (**72**–**75**). Onchitriols show in vitro cytotoxic activity (Rodríguez et al. 1992a,b). Although the absolute stereochemistries of these compounds were established by application of the Mosher–Trost method (Rodríguez et al. 1992a,b), a subsequent total synthesis of onchitriol II (**71**) and of some of its diastereoisomers confirmed the stereochemistry of **71** but suggested that the stereochemistry of onchitriol I (**66**) should be revised (Arimoto et al. 1994). A year later, the total synthesis of onchitriol I confirmed this supposition, allowing the *R* stereochemistry at C-4 and C-10 to be established as represented in **66** (Arimoto et al. 1995).

Finally, from the same New Caledonian collection of *Onchidium* spp, two depsipeptides, onchidin (**76**) and onchidin B (**77**; Fig. 5.9), have been isolated. Both compounds present a new β-amino acid unit. Their structure and absolute stereochemistry were determined by spectroscopic techniques, selective hydrolysis and chiral GC–MS (Rodríguez et al. 1994; Fernández et al. 1996).



onchidin (**76**) onchidin B (**77**)

Fig. 5.9. Depsipeptides from *Onchidium*

5.3.1
Structural Analogy Between Onchidiid and Class II Siphonariid Polypropionates

When onchidiid polypropionates, drawn in ring-opened form, are compared with class II metabolites, a structural trend becomes apparent. PKS enzyme causes the polyketide chain to fold in specific fashions, leading to variations in ring formation patterns. Thus, it appears that a common biosynthetic origin for structurally related metabolites, for instance, siphonarin B (**31**) and the onchidiid ilikonapyrone (**58**), could be explained when the respective polyketide chain precursors folded in a different manner caused by slight differences at reductive and stereochemical levels in their network.

5.4
Secondary Metabolites from *Trimusculus*

Four species of the genus *Trimusculus* have been studied to date and all of them have a common feature: they produce diterpenes belonging to a unique class of labdane skeleton whose only structural variations are the degree and sites of oxidation. The most common functionalizations are acetoxy and isovaleroxy esters (Fig. 5.10).

The first species studied was *T. reticulatus* collected at San Nicolas Island, California. Diterpenes **78** and **79** were isolated from the extracts of both the whole animals and the mucus *T. reticulatus* produces to repel the starfish *Pisaster ochraceus* (Rice 1985) and *Astromei* sp. (Manker and Faulkner 1996). These compounds have a labdane skeleton possessing four contiguous asymmetric carbons on ring B (Manker and Faulkner 1987). The structure of compound **78** was confirmed by total synthesis (Gao et al. 1996).

T. conica collected in New Zealand yielded the diterpene **80** and the steroid **81**. A careful investigation of the localization of the secondary metabolites of *T. reticulatus* from California and *T. conica* from New Zealand indicated that diterpenes **78**, **79**, and **80** were heavily concentrated in the mantle and foot of their respective organisms, while the viscera contained none of the compounds (Manker and Faulkner 1996).

The acetoxydiol **82** was isolated from *T. peruvianus*, collected in the intertidal area of Las Cruces (V Region, Chile; Rovirosa et al. 1992), while four new diterpenes **83–86** were isolated from another collection from El Tabo (V Region, Chile; San-Martín et al. 1996). Compounds **83–86** are the only labdane compounds isolated from *Trimusculus* that possess *Z* geometry on the double bond of the acyclic chain. Compounds **82–86** were evaluated for antimicrobial activity but only compound **84** exhibits modest activity.

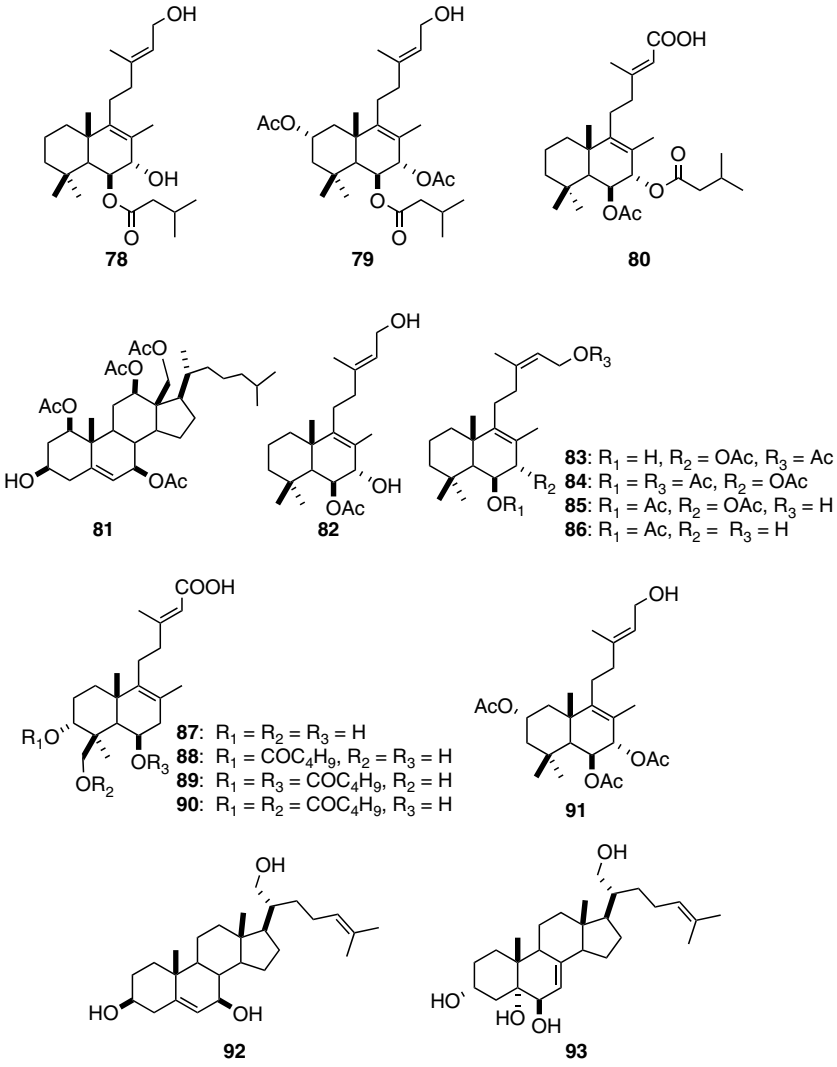


Fig. 5.10. Labdane and steroidal metabolites from *Trimusculus*

From another sample of *T. peruvianus* collected in Antofagasta (II Region, Chile) four new diterpenes were isolated, compounds **87–90**, presenting a new oxidation pattern. The absolute stereochemistry of **90** was established by application of the modified Mosher method. These compounds exhibit in vitro moderate cytotoxic activity (Díaz-Marrero et al. 2003a).

From *T. costatus*, endemic to South Africa, two diterpenes were obtained: **85** isolated previously from *T. peruvianus* and the new compound **91**, both exhibiting antifeeding activity against the predatory fish *Pomadasys commersonnii* and proving toxic to *Artemia salina* (Gray et al. 1998).

Finally, also from *T. peruvianus*, two new steroids, **92** and **93**, were isolated. The Δ^7-3α,5α,6β-triol nucleus of **93** is unprecedented in naturally occurring marine steroidal metabolites. Compounds **92** and **93** possess in vitro cytotoxic activity (Díaz-Marrero et al. 2003b).

Marine pulmonates of the genus *Trimusculus* are unusual not only in habitat and behavior but also in economizing the biosynthesis of diterpenes to a single type of labdane skeleton. Neither carbon rearrangement nor any functional group that induces significant structural modification has been observed in the diterpene metabolites they produce. The selectivity at the oxidation site along the skeleton resembles the mode of action of certain fungi on diterpene substrata, suggesting that some symbiotic microorganism interaction should not be excluded (Díaz-Marrero 2003a).

5.5 Conclusions

Marine pulmonates inhabit the intertidal zone and, with the adoption of fairly elaborate behavioral or chemical defenses against predators, have served as study organisms in chemical, physiological, ecological, and evolutionary research (Hisano et al. 1972; Maeda et al. 1998; Hodgson 1999; Katagiri et al. 2002; Grande et al. 2004a,b). Pulmonates share several synapomorphies with opisthobranchs (both conform to the clade Euthynera) but the monophyly of opisthobranchs with respect to pulmonates remains unclear, according to many phylogenetic hypotheses based on morphological characters (Ponder and Lindberg 1997; Dayrat and Tillier 2002). In most animal taxa, changes to the mtDNA gene order are rare, making these markers useful for higher-level phylogenetics, although one exception might be the gastropod molluscs, where the mtDNA gene order is extremely variable (Rokas and Holland 2000). However, Kurabayashi and Ueshima (2000) found that a unique gene arrangement and highly compact genome organization are shared between opisthobranch and pulmonate gastropods, strongly suggesting their close phylogenetic affinity. Recently, a new phylogenetic hypothesis for Euthyneura was proposed, based on the analysis of primary sequence and the phylogenetic utility of two rare genomic changes (Rokas and Holland 2000). Both sources of phylogenetic information clearly rejected the monophyly of pulmonates, supported so far by morphological evidence. In this analysis, the marine basommatophoran *Siphonaria* was placed within the opisthobranchs and shared with them the insertion of a glycine in the Cox 1 protein; and the marine systellommatophoran *Onchidella* was recovered at the base of the opisthobranch plus *Siphonaria* clade. Opisthobranchs, *Siphonaria* and *Onchidella* shared the relative position of the mitochondrial *trnP* gene between the mitochondrial *trnA* and *nad6*

genes and warranted a more complete analysis of the phylogenetic relationships between opisthobranchs and pulmonates to test the monophyly of each group (Grande et al. 2004a,b).

The identification of structural and stereochemical similarity between siphonariid class I and cephalaspidean metabolites and also the shared polypropionates of siphonariid class II and onchidiid metabolites are in line with the above genetic studies. An interesting feature of the Trimusculidae, however, is that they produce no polypropionates but only isoprenoid metabolites. The labdane diterpenes from *Trimusculus* resemble the labdane-type diterpenoids isolated from opisthobranchs (Ciavatta et al. 1995), supporting their close pulmonate–opisthobranch relationship. Nevertheless, sperm ultrastructural studies of marine pulmonates (*T. costatus, T. reticulatus*) show characteristic heterobranch sperm features. Taxonomically useful differences in the shape and dimensions of the acrosome, nucleus and midpiece occur between the species. These results support the recent decision to transfer the Trimusculidae from the Siphonarioidea to a separate superfamily: Trimusculoidea (Hodgson and Healy 1998). The quite different chemistry of *Trimusculus* and *Siphonaria* appears to support this decision.

The similarity of the biosynthetic pathway within taxonomically distant but related taxa with those of bacterial metabolisms poses the question of whether polypropionates of class I and class II are microbial in origin; and it makes the pairs aglajne-1/siphonarienedione, aglajne-2/siponarienfuranone, and aglajne-3/pectinatone ideal models for the study of natural product symbiosis in molluscs, since those biosynthetic similarities could provide relatively rapid access to invertebrate symbiont genes putatively involved in the biosynthesis of polypropionates (Moffitt and Neilan 2003).

In conclusion, an understanding of how genetic information is correlated with chemical structures would prove useful in regard to secondary metabolites as taxonomic characters and evolutionary markers, as well as providing a valuable tool for designing modifications of the biosynthetic process through genetic engineering (McDaniel et al. 1999), with the goal of producing novel biomedically useful non-natural compounds.

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